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PARASITOLOGY

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EDITED BY

GEORGE H. F. NUTTALL, F.R.S.
Quick Professor of Biology in the University of Cambridge

AND

A. E. SHIPLEY, F.R.S.
Reader in Zoology in the University of Cambridge

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CONTENTS

No. 1 (March).

PAGE

GILRUTH, J. A., SWEET, GEORGINA, and DODD, SYDNEY. Observations on the occurrence in the blood of various animals (chiefly monotremes and marsupials) of bodies apparently identical with *Anaplasma marginale* Theiler, 1910. (With Plate I) . . 1

HUFFMAN, O. V. and WHERRY, WM. B. A description of four *Filaria loa* from the same patient. (With Plate II) 7

DREW, G. HAROLD. A note on the application of Giemsa's Romanowsky stain to the blood and tissues of marine invertebrates . . . 19

PORTER, ANNIE. Some remarks on the genera *Crithidia*, *Herpetomonas* and *Trypanosoma* 22

HINDLE, EDWARD. The passage of *Trypanosoma gambiense* through mucous membranes and skin 24

MACKINNON, DORIS L. On some more protozoan parasites from Trichoptera. (Plate III and 8 Text-figures) 28

NUTTALL, GEORGE H. F. and MERRIMAN, G. The process of copulation in *Ornithodorus moubata*. (With 1 Text-figure) 39

NUTTALL, GEORGE H. F. On the adaptation of ticks to the habits of their hosts. (With 26 Text-figures) 46

BACOT, A. W. The persistence of *Bacillus pyocyaneus* in pupae and imagines of *Musca domestica* raised from larvae experimentally infected with the bacillus 68

HUFFMAN, OTTO V. The embryos of *Filaria loa*. (With Plate IV) . 75

236719

No. 2 (June).

PAGE

DARLING, S. T. The probable mode of infection and the methods used in controlling an outbreak of equine trypanosomiasis (*Murrina*) in the Panama Canal zone 83

SANT' ANNA, JOSÉ F. On a disease in man following tick-bites and occurring in Lourenço Marques 87

NUTTALL, GEORGE H. F. On symptoms following tick-bites in man . 89

SEIDELIN, HARALD. An iron-haematein stain. With remarks on the Giemsa stain. (With Plate V) 94

SWELLENGREBEL, N. H. and STRICKLAND, C. Some remarks on Dr Swingle's paper "The transmission of *Trypanosoma lewisi* by rat fleas," etc. 104

SWELLENGREBEL, N. H. Note on the morphology of *Herpetomonas* and *Crithidia*, with some remarks on "physiological degeneration." (With 12 Diagrams) 108

WOOLLEY, PAUL G. and HUFFMAN, OTTO V. The ova of *Schistosoma japonicum* and the absence of spines 131

HINDLE, EDWARD. The transmission of *Spirochaeta duttoni* . . 133

WOODCOCK, H. M. A reply to Miss Porter's note entitled "Some remarks on the genera *Crithidia*, *Herpetomonas* and *Trypanosoma*" 150

PORTER, ANNIE. Further remarks on the genera *Crithidia*, *Herpetomonas* and *Trypanosoma*, and Dr Woodcock's views thereon . 154

HOWARD, C. W. An experiment in fumigation of ticks . . . 164

MERRIMAN, GORDON. The geographical distribution of *Ornithodorus moubata* (Murray, 1877). (With a Map) 168

MAXWELL-LEFROY, H. Notes on entomological boxes 174

No. 3 (October).

NUTTALL, GEORGE H. F. Notes on Ticks. I. (1) *Ixodes caledonicus*, description of male, together with considerations regarding the structure of the foot in male *Ixodes*. (2) Types of parasitism in ticks, illustrated by a diagram, together with some remarks upon longevity in ticks. (3) Regarding the loss of life in ticks occurring on wandering hosts. (With 2 Figures) . . 175

PAGE

HINDLE, EDWARD. The relapsing fever of Tropical Africa. A Review. (With 2 Maps and 1 Chart) 183

WELLS, R. T. Aerial contamination as a fallacy in the study of amoebic infections by cultural methods. (With Plate VI) . . 204

MCCOY, GEORGE W. A microfilaria (*Microfilaria rosenaui* n. sp.) from the California ground squirrel (*Citellus beecheyi*). (With Plate VII) 220

STRICKLAND, C. Description of a *Herpetomonas* parasitic in the alimentary tract of the common green-bottle fly, *Lucilia* sp. (With Plates VIII and IX and 2 Text-figures) . . . 222

PORTER, ANNIE. The structure and life history of *Crithidia pulicis*, n. sp., parasitic in the alimentary tract of the human flea, *Pulex irritans*. (With Plate X) 237

ACTON, HUGH W. and HARVEY, W. F. The nature and specificity of Negri bodies. (With Plate XI and 2 Text-figures) . . . 255

WENYON, C. M. Oriental sore in Bagdad, together with observations on a gregarine in *Stegomyia fasciata*, the haemogregarine of dogs and the flagellates of house flies. (With Plates XII–XVI and 36 Text-figures) 273

No. 4 (December).

SWELLENGREBEL, N. H. The life-history of *Pleistophora gigantea* Thélohan (*Glugea gigantea* Thél.). (With Plates XVII and XVIII and 20 Diagrams) 345

JOHNSTONE, JAMES. *Tetrarhynchus erinaceus* van Beneden. I. Structure of larva and adult worm. (With Plates XIX–XXIV and 9 Text-figures) 364

LEBOUR, MARIE V. A review of the British marine Cercariae. (With Plates XXV–XXIX) 416

HUFFMAN, OTTO V. The Kurloff-body, a spurious parasite. (With 11 Text-figures) 457

HINDLE, E. On the life-cycle of *Spirochaeta gallinarum*. Preliminary Note. (With 5 Figures and Diagram in Text) 463

ROBINSON, L. E. New species of ticks (*Haemaphysalis*, *Amblyomma*). (With 4 Text-figures) 478

INDEX OF AUTHORS 485

INDEX OF SUBJECTS 487

VOLUME IV MARCH, 1911 No. 1

OBSERVATIONS ON THE OCCURRENCE IN THE BLOOD OF VARIOUS ANIMALS (CHIEFLY MONOTREMES AND MARSUPIALS) OF BODIES APPARENTLY IDENTICAL WITH *ANAPLASMA MARGINALE*, THEILER, 1910.

BY J. A. GILRUTH, D.V.Sc., M.R.C.V.S., F.R.S.E.,
GEORGINA SWEET, D.Sc., AND
SYDNEY DODD, D.V.Sc., F.R.C.V.S.

(*From the Veterinary Institute, Melbourne University.*)

(With Plate I.)

Host animals.	No. examined	No. in which anaplasms present
LIVING.		
Sus scrofa Linn., Common pig.		
Native animals.		
Canis dingo Blumen. (3 months old), Dingo.	1	1
Petaurus breviceps Waterh., Lesser flying "opossum."	2	2
Trichosurus vulpecula Kerr., Common "opossum."	4	4
Pseudochirus peregrinus Bodd., Common ring-tailed "opossum."	2	2
Aepyptymnus rufescens Gray, Rufous rat kangaroo.	4	4
Macropus ruficollis var. *bennettii*, Waterh., Bennett's wallaby.	5	3
Onychogale frenata Gould, Bridled kangaroo.	2	1
Phascolomys mitchelli Owen, Common Australian wombat.	1	1
Tachyglossus aculeatus Shaw, Echidna.	3	2
DEAD.		
Trichosurus vulpecula Kerr.		
Macropus robustus Gould, Wallaroo.		
Macropus dorsalis Gray, Black striped wallaby.		
Macropus ruficollis var. *bennettii*, Waterh.		
Tachyglossus aculeatus Shaw.		

In the blood of the above mammals there was found in varying proportions those enigmatical bodies recently described by Theiler[1] as Protozoa under the name *Anaplasma marginale*, but more generally known as "marginal points" or "coccus-like bodies."

The parasitic character of these masses of chromatin was not regarded as proved by Bruce and others[2] writing somewhat earlier, though still considered possible.

Their importance is considerable in the light of Dr Theiler's claim that they are the specific cause of a cattle disease, transmitted by cattle ticks, to which disease he has given the name of Anaplasmosis, as being distinct from South African Redwater caused by *Piroplasma bigeminum*, though Texas Fever of the Americans is said by Theiler to be a combination of the two diseases now claimed to be separated by him in South Africa. In this connection it is interesting to note that one of us (S. D.) has occasionally noticed the coccus-like bodies in the blood of animals affected with Piroplasmosis in Queensland, but he has not been able to satisfy himself that they were the same as the "marginal points" with which he was familiar in South Africa.

The presence of these bodies, or of bodies indistinguishable from them, has been noticed by Bruce and others[3] in healthy young rats, goats, calves, etc. One of us (J. A. G.) has already recorded the presence of what he now recognises as similar bodies in pigs affected with contagious pneumonia in New Zealand[3] as "small bodies like very short cocco-bacilli and cocci, sometimes in pairs, staining deeply, lying free, but also other bodies within the red corpuscles. These intra-corpuscular bodies were fairly numerous, sometimes six to eight affected corpuscles being in one field. Circular in outline, they varied in diameter from a small coccus to twice the size of an ordinary *Staphylococcus*. They were almost invariably situated to one side of the cell-centre. In smears fixed in alcohol and stained with Giemsa they were more readily observed. They coloured deeply and uniformly with the blue. Frequently a distinct zone, unstained or but very faintly blue-stained, was present between the circular blue-stained bodies and the pink bodies of the erythrocytes. Occasionally two bodies were found within one cell." Some experiments detailed in the above report were carried out which

[1] *Journ. Compar. Pathol. and Therap.* Vol. XXIII. pt. (2), June 1910, p. 108; and *Transvaal Dept. of Agric., Farmers' Bulletin*, No. 111, 1910, p. 7.

[2] *Reports of the Sleeping Sickness Commission of the Royal Society*, No. x. 1910, pp. 100, 101.

[3] *17th Report Dept. of Agric. New Zealand*, 1909, p. 302.

were sufficient to indicate that these bodies could apparently be transferred to other animals of the same species by inoculation and by skin parasites, and further: "media of all kinds inoculated with blood remained sterile, while blood within sterile pipettes placed at blood heat did not alter, and showed no increase, but rather a decrease, of the bodies described."

They were also in February of this year (1910) found by one of us (J. A. G.) in two Victorian pigs which were in an unthrifty condition due to dietetic errors as indicated by their rapid recovery on being supplied with proper food. During the period of observation, seven to ten days, there was no manifest change in the number of anaplasms. They have since also been found in healthy pigs.

The special interest of this note however lies in the discovery of these bodies in the blood of monotremes and marsupials.

General Description.

The bodies found by us in the animals noted above correspond to Theiler's description of *Anaplasma marginale*. With Giemsa's and similar stains they stain a deep blue with clear cut outline, and vary in diameter from 0·9 μ to 2·6 μ. Both intra- and extra-corpuscular bodies are frequent. When within the erythrocyte they may occupy any position, although the great majority are to be found near and sometimes at the margin. The non-staining polar zone is not infrequently found. Double forms, probably fission products, are also to be seen, usually free, but also intra-corpuscular. A smear of blood from one *Pseudochirus peregrinus* shows, in a field of 0·17 mm. containing 27 anaplasms, seven double forms. The number present varies considerably in the different animals but they are quite as numerous in smears from apparently healthy living marsupials and monotremes as in those from dead animals of the same species. In one specimen of *Petaurus breviceps* (immature), besides the usual forms, there were some others (apparently anaplasms) surrounded by a definite clear oval or round capsule (see Fig. 9). The body within this capsule varied in staining reaction, being generally indistinguishable from the ordinary intensely stained anaplasm but at other times faintly stained (with Giemsa's and Jenner's stains) and more or less irregular or wedge-shaped. These encapsuled forms were found both intra-corpuscular and free, the oval forms being 3·5 μ in length and 3·1 μ in width, and the round forms 3·1 μ to 3·2 μ in diameter.

Also in two (living) specimens, a *Petaurus breviceps* and a *Pseudochirus peregrinus*, there were, besides the typical spherical anaplasms, deeply staining elongated pear- or almost wedge-shaped bodies about 1·7 μ in length, both intra- and extra-corpuscular. These were grouped, often with the coccus-like bodies, in a way exactly comparable to those shown by Bruce and others (*loc. cit.*, Pl. X, Fig. 3 in the top five cells).

Significance of the presence of these bodies.

While the majority of the native animals examined, both living and dead, belonged to the Melbourne Zoological Gardens, one (a young living Echidna) was obtained direct from the country, and several of the others were recent arrivals at the Gardens.

All animals of the same species, even within the same pens, do not apparently contain these bodies, *e.g.* of four living *Macropus ruficollis* var. *bennettii* examined two alone showed their presence, and of two *Onychogale frenata* but one was affected. In the only *Petrogale penicillata* available none could be detected.

So far as the Wallabies and the Echidnas are concerned no blood lesions existed, even in those which had died and were examined post-mortem. Unfortunately careful autopsies could not be made owing to the animals being required for anatomical purposes. In any case it is well known that among such animals kept in captivity the mortality is generally very great.

From only one living animal (a *Trichosurus vulpecula*), which was evidently in a state of bad health, were smears obtained, and in these the bodies were found in large numbers. This "opossum" died 39 days later and was brought to the Laboratory some hours after death. There was, in addition to the ordinary blood lesions of anaemia, an unusually great number of normoblasts as well as very numerous coccus-like bodies, very many of which, both free and endo-corpuscular, are indistinguishable from *Anaplasma marginale*, but there were also present numerous cocci and short and long bacilli. This animal was evidently suffering from a marked anaemia and reconstitution of the corpuscles following on a great loss of blood, the intestines being very full of blood. The cause of this haemorrhage could not be determined, there being no sign of physical injury. The examination however was incomplete. The most important point is that there was no definite increase in the number of anaplasms present in the blood, as compared with the number previously found.

In the majority of the other "opossums," both living and dead, there were slight indications of anaemia, such as anisocytosis and poikilocytosis, with polychromatophilia, but whether there was any connection between these blood lesions and the presence of the anaplasms is very doubtful.

The discovery of these bodies in the blood of a very young "pouch" embryo of *Onychogale frenata* 10 cm. long, and of a young three months' old dingo, is of some interest. In the former there were many nucleated red blood corpuscles, and in some cases it is difficult to distinguish between small nuclei and what might be large varieties of anaplasms, but notwithstanding this some cells contain bodies which, in regard to all details, are identical with those described as anaplasms. The young dingo (*Canis dingo*) was born at the Zoological Gardens and had never been out of the cage in which it was born. Although the number of anaplasms, both free and endo-corpuscular, was extremely few yet their nature is undoubted.

On the whole, from the evidence available, we are inclined to the belief that, at least so far as these indigenous forms of mammals are concerned, the presence of these bodies, though possibly parasitic in nature, has no pathological significance.

It should be noted that we have not detected any piroplasms in the blood of any of these animals.

The very great similarity between the staining reaction of the anaplasms and the nuclei of the normoblasts suggests the possibility of their being simply the remnants of the nuclei of normoblasts, which may become extruded from the cell. On the other hand, one cannot but be impressed by the great difficulty, at times, of distinguishing the anaplasm from a coccus.

ADDENDUM.

Since writing the above we have had an opportunity of examining eight *Phascolomys ursinus* (the supposed extinct wombat of Flinders Island, Bass Straits) and of securing blood smears from each. These animals are of various ages, from half-grown upwards, and are in perfect health. All were captured quite recently on the Island, four having arrived only two days before the blood examinations were made. Since arrival in Melbourne they have not been in contact with any other animals, having been kept isolated in special quarters at the Melbourne Museum.

The blood of each of these wombats shows the presence of the bodies as described above, both intra- and extra-corpuscular, single and double forms, varying in size. It should be observed that in no case was the number of anaplasms great, in fact, in two specimens they were only detected after considerable search.

In none of the animals we have examined were any ecto-parasites detected.

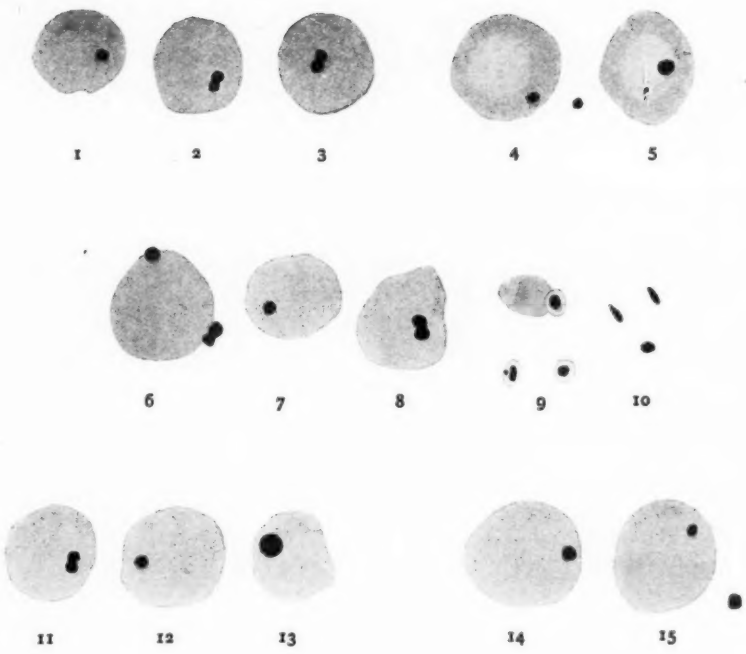
We desire to express our thanks to Mr D. Le Souef, Director of the Zoological Gardens, through whose courtesy we were enabled to make the examinations of animals belonging to the Gardens, and to Professor Baldwin Spencer, Director of the Museum, for the privilege of examining the Flinders Island wombats.

J. A. Gilruth,
Georgina Sweet.

EXPLANATION OF PLATE I.

All figures were drawn under Zeiss Camera Lucida at a magnification of 2200. Affected red corpuscles from five of the animals mentioned above.

Figures 1 to 3 *Tachyglossus aculeatus.*
4 and 5 *Trichosurus vulpecula.*
6 to 10 *Petaurus breviceps.*
11 to 13 *Macropus dorsalis.*
14 and 15 *Sus scrofa.*



G. Sweet. del.

A DESCRIPTION OF FOUR *FILARIA LOA* FROM THE SAME PATIENT.

By O. V. HUFFMAN, M.D.,
and Wm B. WHERRY, M.D.

(*From the Laboratory of the Cincinnati Hospital, Cincinnati, Ohio, U.S.A.*)

(With Plate II.)

A comparison of the descriptions of the morphological characteristics of *Filaria loa* by previous authors shows differences which are explained by the study of our uninjured specimens. We have prepared a table (q.v.) which shows the results of the work of various observers together with our own. We believe that most of the drawings so far published are somewhat misleading; we have, therefore, drawn the essential parts as seen through the microscope with an entire diameter and a portion of a longitudinal section in focus. The drawings are not composite or schematic but have been made on a large scale with accuracy in the measurements so as to represent the different parts in proper proportion (see Plate).

This species of Nematode was first described in 1777 as being different from the Guinea-worm by Guyot, a French naval surgeon, after he had made several voyages to the coast of Angola. He noted that these worms were very white, harder and shorter than the Guinea-worm.

A number of cases of the removal of worms from the eyes of negroes in St Domingo, Cayenne and South America were reported, but it was not until 1838 that the next identification of this species was made when Guyon described a *Filaria* which had been removed from the eye of a negress on the Island of Martinique and sent to him. He also, in

1864, described part of a worm removed from the eye of a negro in Gaboon. In 1877 Morton, of Philadelphia, published an account of a *loa* removed from the eye of a native of Gaboon, with a brief description of the worm by Leidy. The specimen had dried in transit. In this account we also find the first record of infections in Caucasians. Bachelor (1879), of Gaboon, extracted a specimen from the eye of a native and sent it perfectly preserved to the United States. Leuckart[3] (1886) identified the species as distinct from the Guinea-worm; he had received a female 41 mm. long.

In 1895 Manson[6] described a male *Filaria loa* removed from the eyelid of an Englishman who had been in West Africa; he also described a male and a female removed from the eye of an English lady who had been in Old Calabar. In the same year Ludwig described a female specimen removed from the eye of a Russian who had been in Fernando Po, Gaboon, Kamerun and the Gold Coast. The specimen had been injured in several places. Blanchard (1899) gave an extended account of the anatomy of a male and a female removed from the same host, who had been in the French Congo; these worms were quite young. In 1901 Annett, Dutton, and Elliott obtained a female *Filaria loa* at Bonney but they did not report in regard to the host nor from what part of the body it was obtained. In 1903 two males and two females, sent from Nigeria, were briefly described by Ozzard. Looss[5] (1904) published a complete anatomical description of two specimens which had been sent to him from the Gold Coast and labelled *Filaria loa* without other data. The male was very slightly injured by forceps just behind the cephalic end. Ward[9] (1906) noted some points which he considered peculiar to the three males studied by him and referred to the admirable account of Looss for a complete description of their structure. One of his specimens had been sent to him mounted in balsam. This author gives a most complete critical bibliography on the morphology of *Filaria loa* as well as on all cases reported. He also calls attention to the cases which have been recorded and wrongly assigned to *Filaria loa*.

In the same year Livon and Pénaud[4] (1906) observed a female but did not describe it. They report more fully in regard to the microfilariae observed in the blood, urine, and saliva of the host as well as those which were hatched from eggs which had been artifically removed from the adult female. Later Billet[1] (1906) described a male 24 mm. × 0·4 mm. He states that he agrees with Manson, Bernard, Blanchard, Ozzard, Penel, and Wurtz. He does not refer to Looss'

description, and he gives but few measurements and no drawings. Where he cites Bernard as an authority we must call attention to the fact that the male *Filaria loa* reported by Bernard was identified by Blanchard.

Wurtz and Nattan-Larrier[10] (1907) reported a new observation of *Filaria loa*, but they did not describe the *Filaria* which was removed from the eye nor did they exclude other diseases which might have caused the various symptoms shown by their patient.

Thus, with the aid of Ward's critical bibliography up to Jan. 1st, 1906, and a review of the literature since then, we have gone over the various descriptions of *Filaria loa*, and these show the necessity of recording further descriptions of the adult form of this species.

The specimens examined by us were kindly placed at our disposal by Dr Emil Blunden, of Cincinnati, who had removed them himself from his wife's eyes. The infection was apparently contracted in 1907 outside of Batanga, Cameroon, West Africa. In all seven *loa* have been extracted to date. Our specimens comprise two females extracted in Africa; a male, and one female, which had been broken off 20 mm. back of the head, extracted in America. The first three specimens were in perfect condition and had been preserved in 10 % chloral hydrate. Before examination they were placed in 70 % alcohol containing 5 % glycerine heated to 60° C. These uninjured specimens were of a brownish yellow colour and became sufficiently clear for the purpose of studying their internal structure. The thread-like body of each remained in a slightly irregular, compound curve. Each worm without any stretching and without difficulty was temporarily placed upon a glass slide and covered with alcohol-glycerine solution sufficient to support a cover slip. In this way the worm could be turned and observed from every aspect. We paid considerable attention to those structures which have been variously described by others. The fourth specimen had remained in tap-water for 48 hours. It did not become clear and remained very white.

Before describing each of these specimens separately we will mention wherein our observations differ from those of previous authors: we do not find the bosses on the cuticle to be so high as previous observers found them. Their average height upon our specimens is equal to one half the thickness of the cuticle. We find the cuticle thicker than that of the specimens either of Looss or of Blanchard. Ludwig reported the cuticle as thicker than we do but he was working at a female specimen which had been injured and partly emptied of its intestine and genital

organs. Like Ludwig, and differing from Looss, we find very delicate transverse striae in the tail of the female. In one female (Fig. 3), the posterior oviduct passes to the very tip of the tail, and these striae are not seen so clearly as in the other (Fig. 5) wherein the posterior oviduct turns upon itself to pass forward without entering the curved portion of the tail. Naturally in the latter more light may pass through the tail, making careful observation of both layers of the cuticle less difficult, and thus accounting for the above difference between Ludwig and Looss because in the former's specimen the posterior oviduct did not pass back into the curved portion of the tail while in the latter's specimen it did.

Like Ward's and Billet's but differing from that of Looss' our male presents a distinctly well-curved tail (Fig. 8). In regard to the circumanal papillae of the male and their arrangement we, like Manson, do not find such marked asymmetry that we should lay stress upon it. When we carefully observed the papillae from the ventral aspect they appeared symmetrical but one might easily judge those on the left side to be larger and less close together. We do not find anything to indicate the appearance of orifices on the summits of the largest pair of papillae as Billet did. Like Ozzard, we find the anus of the female at a greater distance from the tip of the tail than does Looss.

In the main we can corroborate Looss' anatomical descriptions. Probably through a typographical error Looss refers to the embryo as 0·035 mm. to 0·37 mm. × 0·022 mm. The 0·37 mm. should be 0·037 mm. We were fortunate enough to observe some free embryos (Fig. 6) and like Ludwig we find them to have distinct sheaths. This seems important as Manson at one time did not believe the embryo had a sheath.

The drawings or sketches of *Filaria loa* copied in most treatises on parasites are generally misleading. The spicules are represented as of similar size and disproportionately long. The cuticular bosses and circumanal papillae are represented disproportionately large. The bosses, sometimes called tubercles or buckles, appear to us to be elevations of a portion of the outer layer of the cuticle and their contents are absolutely clear and do not show any cellular structure.

At this point we should call attention to the fact that the vulva is not at the junction of the anterior one-fourth with the posterior three-fourths of the body as erroneously stated in *The Animal Parasites of Man* (Braun[2], 1906). Ludwig is responsible for this mistake as he could not find the genital opening of the female and decided that it

was in that portion of his specimen which had been mutilated. He also mislocated the anus.

Scheube [8] (1903) states that the *Filaria loa* female rarely attains the length of 70 mm. Maurel's case, reported by Trucy in a paper on the Guinea-worm, is responsible for this statement. Scheube is also in error when he refers to GUYOT 1838 under the Literature. It should be Guyon. (Ward, 1906.)

Description of our Specimens.

The Male.

External structure. The male is 35 mm. long. The tip of the anterior extremity is 0·056 mm. in diameter. At the centre of the tip is the oral opening. At the base of the cephalic cone, which is 0·07 mm. from the tip, it is 0·14 mm. in diameter.

From the base of the cephalic cone the body gradually increases in diameter until it reaches its greatest circumference with a diameter of 0·35 mm. at a distance of 0·7 mm. from the anterior end. This maximum diameter of 0·35 mm. is maintained throughout the remainder of the anterior one-sixth of the entire body length whence it gradually and evenly diminishes in circumference down to the rounded tip of the posterior extremity. The posterior extremity is curved ventrally like a shepherd's hook and presents the ano-genital opening on its ventral aspect, 0·112 mm. distant from the tip. At the ano-genital opening it is 0·168 mm. in diameter.

The cuticle is 0·012 mm. thick. At the extremities it gradually becomes reduced in thickness reaching a minimum of 0·008 mm. It consists of an outer harder layer and an inner softer layer. With the exception of that covering the anterior extremity for a distance of 3·5 mm. from the oral opening and the posterior extremity for a distance of 1·19 mm. from the tip of the tail the cuticle presents, at irregular distances, bosses which arise from the outer layer of the cuticle. These bosses are not hemispherical. Their average diameter at the base is 0·018 mm., and their average height is 0·006 mm. or one half the thickness of the cuticle.

On the ventral aspect of the posterior extremity there are five pairs of papillae. The anterior pair is the largest. They successively decrease in size from the anterior to the most posterior pair which is almost at the tip. The ano-genital opening is partially between but mostly

posterior to the third pair. If we could not discern the interior structure we should simply note slight undulations of the cuticle instead of papillae.

The cuticle is non-striated with the exception of that covering the posterior extremity. Here transverse striae are perfectly discernible and, by focussing, they may be seen to extend across the entire width of the body at this portion. Upon close observation it may be noted that the outer and thinner epiderm is not striated.

Internal structure. Beginning at the oral orifice is the oesophagus, formed by large cylindrical cells. On either side there is a dark granular mass due to nerve cells and probably to some glandular elements. For a distance of 0·2 mm. the lateral bands and the powerful musculature may be observed with considerable clearness when the beginning of the intestine, the testicle, and greater thickness of the body make the internal parts less easily discerned. However, the intestine and the testicle can be determined throughout the entire body. In the anterior part the testicle is composed of spermatoblasts of polyhedral shape. Lower in the posterior portion free spermatozoa occur. 0·114 mm. above the ano-genital opening when viewed from the left side we observe the upper thicker end of one of the spicules. It appears to be transversely concave, and transversely striated near its upper end. Upon close study we are not sure but that these coarse striae are caused by the spermatozoa in the adjoining ejaculatory duct where they occlude the light from passing through the highly refractile and dense edges of the chitinous spicule. The spicule gradually diminishes in size and curves towards the anal opening just before reaching which it is lost to view. Neither spicule projects from the ano-genital opening. It is 0·28 mm. from the tip of the posterior extremity of the body to the base or anterior end of the longer spicule.

Upon turning the worm over and examining it from the right side a shorter spicule, like the lower end of the long one, is seen partially crossing the longer one just inside the ano-genital opening.

Anterior to the anus there are three of the five pairs of papillae. They are distinct pyriform masses projecting from the musculature and cause the papillae noted when examining the cuticle. These papillae do not protrude from the skin so much as their interior granular portions protrude as distinct pyriform projections from the musculature. Billet suggests that the largest pair may give rise to a secretion of some aid to the act of copulation. The anterior pair are situated

anteriorly to the ano-genital opening at a distance equal to the length of the post-anal portion of the tail. This pair is the largest and most perfectly pyriform. The pairs are successively smaller and situated closer together. Between the two rows of papillae there is a slight concavity but the ano-genital opening is at a slight elevation.

The Females.

External structure. The first female examined is 50 mm. long and 0·5 mm. at its greatest diameter. It is 0·084 mm. across the tip of the cephalic cone. The base of the cone is 0·084 mm. from the tip, and at this point the diameter is 0·182 mm.

At a distance of about 0·7 mm. from the anterior end the maximum diameter of 0·5 mm. is reached. This diameter is maintained throughout the anterior one-fifth of the entire body length whence it gradually and evenly diminishes in circumference back to the rounded tip of the posterior extremity, which is partially curved ventrally but not so much as the tail of the male.

The anus is 0·21 mm. from the tip of the posterior extremity on the ventral aspect. At the anus the diameter of the body is 0·192 mm.

The cuticle is of the same thickness as in the male. At 2·1 mm. from the anterior tip is the genital opening, which causes a slight protuberance.

The first boss is 4·2 mm. from the anterior end. From here on the bosses occur at irregular intervals and are more numerous than in the male. Furthermore, they are found upon the posterior extremity back to the very tip.

In the slightly curved posterior portion the cuticle is striated, but not so plainly as in the male. This appearance may be due to a crimping of the inner softer layer yet it shows most distinctly beyond the anus where there is no bending of the tail.

Internal structure. The cephalic cone is translucent as the heavy musculature does not project into it. At its base and along the oesophagus there are dense granular masses which may be glandular or nervous tissue. These obscure the light to such a degree that we cannot discern any papillae about the head.

At 0·35 mm. posteriorly we come to a loop of the anterior oviduct. Within the oviduct we can discern numerous ova or embryos. The oviduct is 0·07 mm. in diameter and does not cross itself anteriorly to the vulva. The oesophagus and intestine are pushed aside. The musculature occupies a considerable volume at this point.

The vagina curves backward a short distance (0·2 mm.), then suddenly inwards and is lost to view on account of the opacity of the worm. Just before we come to the curve in the posterior extremity the posterior oviduct bends upon itself and we can discern numerous large ova. The intestine follows the greater curvature until about opposite the anus when it can be seen distinctly crossing from the dorsal wall to the anus.

The second female examined is 55 mm. long and 0·5 mm. wide, and presents these differences: 0·49 mm. from the anterior tip we come to the loop of the anterior oviduct that completely crosses itself anteriorly to the vulva which is 1·75 mm. from the tip of the head. The vagina runs parallel to the wall directly from the vulva backwards a distance of 5·6 mm. where it becomes a bifid uterus. It presents thick walls. Near the posterior extremity the two loops of the posterior oviduct seem to fill the entire body cavity and the ova or embryos are not so closely packed. The larger ova measure 0·015 mm. × 0·024 mm. and appear as coiled embryos. At this same part the intestine is discernible as well as two loops of the posterior ovary, which overlie the posterior oviduct in braid-like fashion. In this female the posterior oviduct passes back into the very extremity of the tip where larger ova are discernible. The anus is 0·210 mm. from the tip of the tail. The diameter at the anus is 0·182 mm.

The third female examined had been broken off about 20 mm. from the head. This specimen was white and did not become clear when treated with the alcohol-glycerine. It had been placed in ordinary tap-water and was not preserved, nor were the embryos examined until 48 hours later. The embryos were motionless and probably dead.

In so far as it could be examined it was like the second female. It was from this worm that the free *embryos* were obtained. They occurred in great numbers. Only two were measured. One is 0·279 mm. long, including the sheath projecting from the head end. The sheath projects beyond the head 0·009 mm. The greatest diameter is 0·006 mm. The other is 0·294 mm. long × 0·006 mm. broad. In both the sheath projects beyond the point of the tapering tail 0·015 mm. The anterior end is bluntly rounded while the tail is finely drawn out. The cuticle is smooth and the interior of the body appears to be finely granular. We were unable to observe the V spot.

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Observer	Year	Sex	Length	Maximum width	Diameter at tip of anterior extremity	Diameter at anus	Distance of anus from tip of tail	Distance of vulva from tip of anterior extremity	Distance of first boss from tip of anterior extremity	Distance of last boss from tip of tail	Length of longer spicule	Number of papillae near anus	Thickness of cuticle	Height of skin boss
Leuckart	1881	♀	41*											
Manson	1895	♂	25 to 30	0·3					1·5	1·5		5 prs. sym. 4th pr. adanal		0·00
	1895	♀	35·5	0·5										
Ludwig	1895	♀	41	0·5			?	?	3	last at tip			0·018	0·00
Blanchard	1899	♂	22	0·435			0·082		4·4	0·18		5 prs. asym. 4th pr. postanal	0·009	0·00
	1899	♀	20	0·54			?			last at tip				
Ozzard	1903	♀	55	0·55		0·275	0·3	2·35		0·6				
	1903	♀	50											
	1903	♂	30			0·175	0·175					Same as previous authors		
	1903	♂	35											
Looss	1904	♂	33	0·4	0·05		0·084		2·5	1·2	0·176	5 prs. asym.	0·009	0·00
	1904	♀	52	0·5	0·07	0·17	0·17	2·4		last at tip				
Ward	1906	♂	16											
	1906	♂	22											
	1906	♂	25											
Huffman and Wherry	1910	♂	35	0·35	0·056	0·168	0·112		3·5	1·19	0·168	5 prs.	0·008 at extremities, 0·012 except at extremities	0·00
	1910	♀	50	0·5	0·084	0·192		2·1	4·2	last at tip				
	1910	♀	55	0·5		0·182								
	1910	♀												

* Measurements given are millimetres or fractions thereof.

Height of skin boss	Diameter of base of skin boss	Transverse striae	Size of coiled embryos	Size of free embryos	Infection acquired	Host	From what anatomical locality	Remarks
					Loango	European	Eye	
0·004	0·012				W. Africa	,,	,,	
			0·030	0·25	Old Calabar	,,	,,	Tail extremity not curved.
0·007	0·011 0·018	Very delicate at tail	0·045 × 0·025	0·253 × 0·005	Gaboon Kamerun Gold Coast	,,	,,	Specimens injured in several places. Intestine and genital organs partially protruding.
0·009	0·02 0·027	No striae			French Congo	,,		
					,,	,,		Eggs, no embryo. Cephalic cone less and not so clear as in male. No head papillae.
					Nigeria			
					,,			
					,,			Tail not sharply curved as female. Spicules do not protrude. Short posterior spicule. Longer anterior spicule.
					,,			
0·009		At tail	0·035 × 0·022	? × 0·0047	Gold Coast	?	?	Spicules partially protrude.
		None			,,	?	?	
					Batanga			Tails curved. Spicules protrude.
					,,			
					Africa			
0·006	0·018	Very delicate at tail			Batanga	American	Eye	Tail curved. Spicules do not protrude.
		,,	0·015 0·024		,,	,,	,,	Tail not curved as much as male.
		,,			,,	,,	,,	Tail not curved as much as male.
				0·006 × 0·279	,,	,,	,,	Only anterior 20 mm. preserved.

DESCRIPTION OF PLATE II.

Filaria loa, Guyot.

Fig. 1. Anterior extremity of female showing cephalic cone, oesophagus, loop of anterior oviduct containing eggs and coiled embryos, genital opening, and most anterior boss on cuticle.

Fig. 2. Portion of body of female in posterior one-tenth showing loops of posterior ovary above the posterior oviduct which is filled with coiled embryos.

Fig. 3. Posterior extremity of female in which posterior oviduct passes to the extreme tip before turning upon itself.

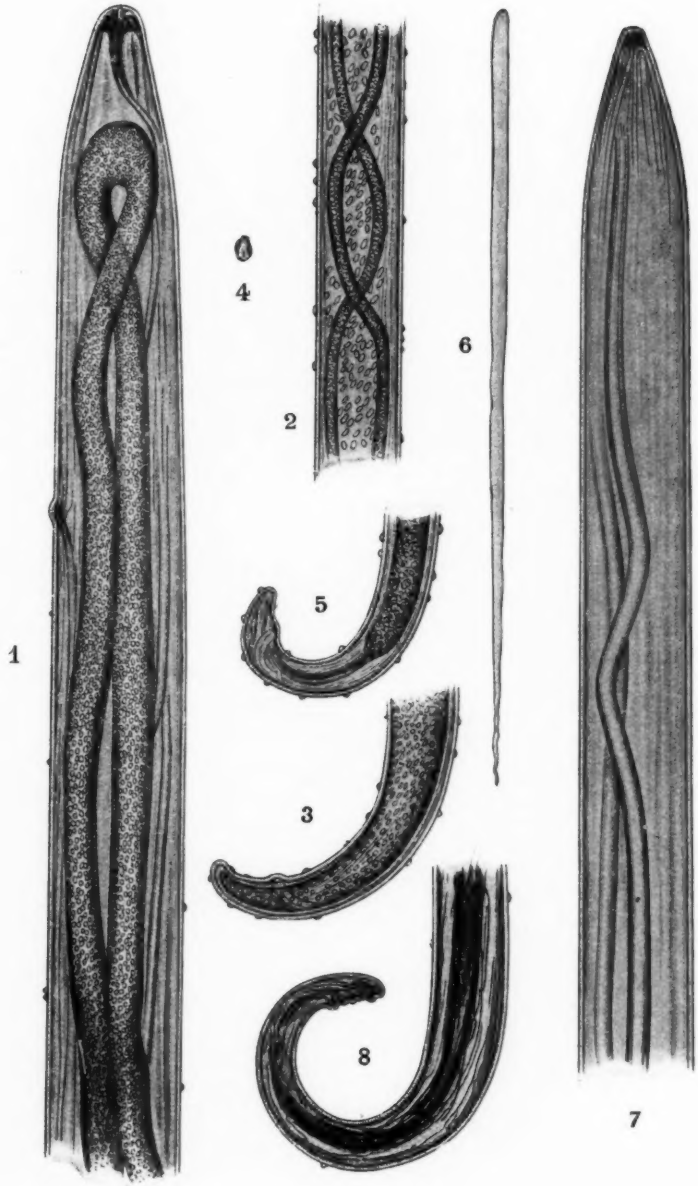
Fig. 4. Coiled embryo 0·015 mm. × 0·034 mm. Much enlarged.

Fig. 5. Posterior extremity of another female in which the posterior oviduct turns forward before reaching the curved portion.

Fig. 6. Embryo much enlarged. Sheath not represented.

Fig. 7. Anterior extremity of male showing cephalic cone, oesophagus, intestine and testicle.

Fig. 8. Lateral aspect of posterior extremity of male showing striae in inner layer of cuticle, last boss, five of the papillae, anus, and the edges of the longer spicule represented by two black lines just anterior to the row of papillae. The black intestine is clearly represented in the body and upper portion of the tail.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A NOTE ON THE APPLICATION OF GIEMSA'S ROMANOWSKY STAIN TO THE BLOOD AND TISSUES OF MARINE INVERTEBRATES.

By G. HAROLD DREW.

Beit Memorial Research Fellow.

THIS method is especially applicable to the blood and body fluids of Invertebrates in which true clotting does not occur, and in which the proportion of proteid substances in the serum is not high.

In the case of marine animals, dry smears of the blood cannot be successfully obtained, as the quantity of salts in solution in the blood prevents that almost instantaneous drying which is necessary, and also gives rise to the formation of crystals which causes much distortion of the organised elements of the blood. Accordingly films of blood must be fixed while wet, and as Giemsa's stain gives the most satisfactory results after Sublimate fixation, the following method was employed:

1. The blood is withdrawn from the animal with a fine pointed glass pipette, the end of which has been carefully rounded in the flame, and two or three drops are allowed to run on to the middle of a slide. It is essential that both pipette and slide be absolutely chemically clean, and should show no scratches or rough surfaces.

2. The blood is allowed to remain on the slide for two or three minutes to allow the corpuscles to settle on the glass, and then an equal volume of a saturated solution of Corrosive Sublimate in sea water is added, care being taken that the fluid on the slide spreads as little as possible. By this means the corpuscles are partially fixed, and are found in a thin film adhering to the glass. Proteid substances in the blood are precipitated, but if not present in large quantities the precipitate does not interfere with the preparation, as little of it adheres to the slide.

3. After ten minutes, the slide is placed in Schaudinn's solution (saturated solution of Corrosive Sublimate two parts, absolute Alcohol

1 part), and may be left in this for twelve hours with advantage. If preferred the fixation may be completed in aqueous Sublimate solution, but then the slide must be treated for some time with 70 % Alcohol, as otherwise the corpuscles are liable to become detached from the glass during staining.

The remaining processes are the same as those given by Giemsa (*Deutsche med. Wochenschr.* No. 40. 1909, and No. 12. 1910) for the application of his stain to sections.

4. The slide is washed in several changes of distilled water and then placed for ten minutes in the following solution

Potassium iodide	2 gms.
Lugol's solution	3 c.c.
Distilled water	100 c.c.

(Lugol's solution consists of KI 6 gms., Iodine 4 gms. distilled water 100 c.c.) I consider that the ordinary Gram's Iodine solution acts equally as well as this mixture.

5. After washing for a short time in water, the slide is placed for ten minutes in a 0·5 % solution of Sodium thiosulphate, then washed for five minutes in tap water, and for five minutes in several changes of distilled water.

6. It is then stained for from two to twelve hours in a solution of one drop of Giemsa's solution (Grübler) to 1 c.c. of distilled water. It is essential that this distilled water be as neutral and as pure as possible. As a rule ordinary distilled water cannot be used as it often contains organic acids and other impurities. Such water should be treated with Potassium bichromate and Sulphuric acid, and re-distilled in an "all glass" still and condenser.

A fresh solution of the stain may with advantage be used after the first half hour.

7. After staining, the slide is rinsed in re-distilled water, and differentiated in the following solutions

1. Acetone 95 parts, Xylol 5 parts.
2. Acetone 70 „ Xylol 30 „
3. Acetone 70 „ Xylol 30 „
4. Xylol, pure.

It is necessary that the Acetone and Xylol be pure and absolutely neutral. Finally the slide may be examined in Cedar Oil, or mounted in neutral Balsam in Xylol. This neutral Balsam solution can be

specially obtained from Grübler & Co.: the ordinary Balsam in Xylol solution as used in most Laboratories will generally be found so acid that it will readily turn blue Litmus paper red, and is quite useless for this purpose.

By this method a very fine differential staining of the corpuscles can be obtained, and such structures as "eosinophil" and other granules in the corpuscles are brought out with remarkable clearness. It has also the advantage that parasites and bacteria present in the blood take the stain well. On the other hand, any method in which the blood is removed from the body, and placed on a slide before fixation, is bound to result in a certain amount of distortion, and often agglutination, of the corpuscles. The only way to avoid this is to fix the corpuscles while still contained within their blood vessels, and this can be done by cutting out pieces of tissue, as small as possible, which contain lacunar blood spaces. Such pieces of tissue should be fixed for from 24 to 48 hours in Schaudinn's solution, passed through the alcohols (without preliminary washing), embedded in Paraffin, cut into sections not exceeding 4μ thick, taken down into distilled water, and then treated in the way described in paragraphs 4, 5, 6 and 7 according to the instructions given by Giemsa. Though by this process so much distortion of the corpuscles is not produced, still in many ways it is not so satisfactory as the former method. The blood cells are not expanded, and are often crowded together so that it is more difficult to distinguish details of structure, granules, etc. Though Giemsa's stain, as applied to sections generally, does not give remarkably good nuclear images, yet it gives a degree of colour differentiation between various tissues, presumably corresponding to chemical differences in these tissues, which is frequently of great value, and it may often with advantage be employed for this purpose, in addition to its more specific use as a stain for protozoon parasites.

SOME REMARKS ON THE GENERA *CRITHIDIA*, *HERPETOMONAS* AND *TRYPANOSOMA*.

By ANNIE PORTER, D.Sc., Lond.

Quick Laboratory, Cambridge, and University College, London.

In this brief note it is not my intention to enter into detailed criticism, but merely to express my surprise and that of other protozoologists, that the personal opinions of the Recorder should be allowed insertion in the *Zoological Record* (1909) which is also the International Catalogue of Zoological Scientific Literature. Against this procedure I beg to record an emphatic protest.

The facts are as follows: On page 60 of the Division *Protozoa* of the *Zoological Record* (International Catalogue) we find "*Crithidia*" *hystrichopsyllae*, Mackinnon; "*C.*" *melophagi*, Swingle; "*C.*" *nycteribiae*, Chatton; "*C.*" *simuliae*, Georgewitch; "*C.*" *tabani*, Patton, all described as "parasites [which] are most probably really trypanomonad phases of a Trypanosome."

Again, on the same page we find "*Herpetomonas*" *ctenophthalmi* Mackinnon described as "probably really a phase of a Trypanosome," and on page 42 as "probably really Trypanosome phases from the mole." The inverted commas enclosing the generic names *Crithidia* and *Herpetomonas* are those of the Recorder, Dr H. M. Woodcock.

At the present time there is not a particle of definite evidence that any of the above-mentioned flagellates (species of *Crithidia* and *Herpetomonas*) are developmental phases of a Trypanosome. Before making remarks in an International Catalogue, the Recorder should bring forward evidence in support of his statements, and it should be unnecessary to remind him that caution in making or even suggesting general statements is one of the marks of the exact thinker. Incidentally, it is rather extraordinary that in a Catalogue the personal

views of the Recorder should be allowed access at all. I beg leave to bring this procedure to the notice of the authorities responsible for the issue of the International Catalogue.

I am not aware that Dr Woodcock has ever published any original observations on species of *Crithidia* or *Herpetomonas*. It will be remembered that Dr Woodcock was the author of a recent article on the Haemoflagellates (1909) which was much criticised at the time of its appearance. It is quite unnecessary to refer further to this matter. Also, I am aware that Dr Woodcock has quite recently (Nov. 1910) published a very lengthy paper on "Studies on Avian Haemoprotozoa," wherein (p. 713) he suggests, but does *not* prove, that *Crithidia melophagia* is "simply a developmental phase of a sheep trypanosome in its alternate, insectan host." It remains for Dr Woodcock to prove this statement experimentally. He will find the researches of Flu, Swingle and Porter directly opposed to his view. Surely he is aware that *Crithidia* and *Herpetomonas* have been described from water-bugs and certain flies which are *not* bloodsuckers.

Finally, may I again be permitted to state that it is out of place for the Recorder to insert his personal opinions in an International Catalogue of Scientific Literature, more especially when his opinions are extreme and decidedly opposed to the opinions of those who have worked personally on the parasites in question.

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THE PASSAGE OF *TRYPANOSOMA GAMBIENSE* THROUGH MUCOUS MEMBRANES AND SKIN.

By EDWARD HINDLE, Ph.D., A.R.C.S., F.L.S.,
Magdalene College, Cambridge: Beit Memorial Research Fellow.

(*From the Quick Laboratory, University of Cambridge.*)

Although in nature Sleeping Sickness is chiefly transmitted by *Glossina palpalis*, the possibility of other modes of infection has been suggested by various authors.

In 1907 Koch brought forward evidence which seemed to show that this disease may be transmitted by coitus, for, in his report from Uganda, the following note occurs: "All attempts to find *G. palpalis* in that part of Kisiba in which the disease chiefly occurs have been proved unavailing; it may now be assumed to be certain that the fly does not occur in those regions." Here 15 infected women were found. "These had never left Kisiba and could only have been infected there. It is, however, further established that the women are all married, that their husbands either have died of Sleeping Sickness, or are suffering from the disease. Of particular importance for the significance of this occurrence of Sleeping Sickness in married women is that in one case a man with trypanosomiasis has three wives and that all three also suffer in a demonstrable manner from trypanosomiasis; one of them is already very ill. *From this it must be concluded that the infection in this case can have been brought about only by sexual intercourse.* If any other agency, *e.g.* bloodsucking insects, were to convey the disease in Kisiba from infected persons to healthy not only women whose husbands suffer from trypanosomiasis must have fallen ill, but also women with healthy husbands, unmarried women, moreover children and older people who live in close contact with sick." (Quoted from *Sleeping Sickness Bulletin*, I. pp. 72—73.)

It is the opinion of most investigators, however, that further evidence of such transmission is necessary before we can consider it definitely proved, and with a view to testing its possibility, the writer has performed various experiments to see if *T. gambiense* is capable of passing through a sound mucous membrane and also the undamaged epidermis.

Numerous authors have carried on various feeding experiments with other species of trypanosomes, with somewhat contradictory results. Whereas on the one hand Musgrave and Williamson[1] carried on feeding experiments with Surra for more than a year, with "hundreds of animals," and obtained no infection, Manteufel[2], working with *T. lewisi*, always obtained infection by introducing infected blood into the mouths of rats. The latter author also showed that *T. lewisi* is capable of passing through the skin of rats and infecting them, but up to the present no similar experiments with *T. gambiense* have been recorded.

The following experiments, therefore, have been performed with a view to ascertaining whether *T. gambiense* behaves in a similar manner to *T. lewisi* as regards passage through mucous membranes and the skin:

(*a*) *Infection per os.* A number of experiments were performed by feeding uninfected rats with the organs of animals which had died of Sleeping Sickness. Although five rats were fed in this fashion, none of them became infected, and thus, at first, it appeared as if infection by feeding could not be effected.

A further series of experiments were made, however, and instead of feeding the rats with the organs of animals which had died of trypanosomiasis, 1 c.c. of infected blood was introduced into the mouth of each rat by means of a syringe. Under these conditions the rats always became infected after an incubation period of from 7—10 days. Great care was taken to prevent the possibility of producing lesions in the mouths of the rats used, and there can be no doubt that the trypanosomes made their way into the blood through the mucous membrane of the alimentary canal.

The contradictory results obtained by feeding with the organs of infected animals and infected blood mixed with food, seems to be due to the effect of the digestive fluids on the trypanosomes. When solid matter

[1] Musgrave and Williamson, *Bulletin No.* 3, *Bureau of Government Laboratories* Manila, P. I.

[2] Manteufel, *Arb. a. d. Kais. Gesundh.* 1909, vol. XXXIII. pp. 46—83.

is given to the rats, there will be a more copious secretion of gastric juice and also fewer parasites introduced, than when blood is injected into the mouth, and consequently there will be a greater chance of the trypanosomes being overcome before they have been able to penetrate the wall of the alimentary canal. It was found by experiment that trypanosomes when mixed with gastric juice soon become non-motile, and the rapidity with which this takes place depends upon the relative proportions of the trypanosome-containing blood and the gastric juice.

(*b*) *Infection per vaginam.* A few experiments were made to ascertain whether *T. gambiense* can be transmitted from rat to rat by coitus, but although infected males were kept with healthy females and *vice-versâ*, up to the present we have not obtained any infection by these means. In addition a number of female rats were isolated and into the vagina of each a drop of infected blood was introduced by means of a small and carefully rounded glass tube, great care being taken to avoid the possibility of producing any abrasions. After incubation periods varying from 5—8 days all the rats became infected and the disease followed its normal course. This experiment has been repeated six times, in every case with positive results.

(*c*) *Infection per cutaneam.* The following experiment was carried out to ascertain if *T. gambiense* could pass through an undamaged epithelium. A drop of infected blood was spread over some part of the body of a rat, whose hair had neither been shaved nor cut, and allowed to dry. After about one hour the dried blood was covered with a layer of collodion, and the rat then set free. After a normal incubation period three (two males and one female) out of five rats thus treated became infected. On repeating these experiments with rats that had been shaved success was invariable, but in the operation of shaving there is always the possibility of producing small abrasions.

The foregoing experiments clearly show that in rats *T. gambiense* is able to penetrate a sound mucous membrane and also an undamaged skin and there is no reason to suspect that this parasite would not possess similar powers in its normal host, man. Moreover, the ease with which it passes through the mucous membrane of the vagina, shows that there is every possibility of Sleeping Sickness being transmitted by coitus.

Although there is no direct evidence to show that trypanosomes ever occur in the seminal fluid, yet in the case of dourine (*T. equiperdum.*) transmission normally takes place by coitus and it is necessary

to assume, either the presence of lesions in the infected partner, or the occurrence of trypanosomes in the seminal fluid.

The experiments recorded above show that in the case of *T. gambiense* there is no necessity for any lesion in the mucous membrane of the healthy partner for infection to take place, once the parasites are brought in contact with it. In restricting the spread of Sleeping Sickness it is important, therefore, to insist upon the necessity for personal prophylaxis, even though infection other than by *Glossina palpalis* (and possibly *G. morsitans*) is probably exceptional.

Note:—Since this paper was written Martin and Ringenbach[1] have published an account of similar experiments with *T. gambiense* in guinea-pigs. These authors obtained infection by introducing infected blood into the vagina of guinea-pigs and also by rubbing infected blood on the shaved skin. They failed to obtain infection, however, by spreading blood on the unshaven skin, except in the case of two females, in which the writers consider that the infected blood ran down the belly and entered the vagina of each of the animals. The experiments recorded above show that, in the case of rats, *T. gambiense* may pass through the unshaven skin, for the experimental conditions were such that it was impossible for the infected blood to come in contact with any mucous membrane.

[1] *Bull. Soc. Path. Exot.* 1910, pp. 433—437.

ON SOME MORE PROTOZOAN PARASITES FROM TRICHOPTERA.

By DORIS L. MACKINNON, B.Sc.
(*Assistant in Zoology, University College, Dundee.*)

(Plate III and 8 Text-Figures.)

Introduction.

In a recent paper (*Journal of Parasitology*, 1910, III. pp. 245—54) I described a new trichomonad (*Trichomastix trichopterorum*)[1] and a spirochaete from the hind-gut of certain trichopterous larvae. I also mentioned the occurrence along with these parasites of another peculiar flagellate, which, at the time, I was disposed to consider as closely related to *Macrostoma caulleryi*, Alexeieff.

The present paper deals chiefly with this flagellate, which seems to be an entirely new form, and for which, after more careful study, I am reluctantly obliged to erect a new genus, *Embadomonas*.

It is extremely interesting to find that the caddis-fly larvae also sometimes harbour the flagellate, *Crithidia campanulata*, Léger, and a microsporidian which I regard as *Gurleya légeri*, Hesse. The first of these has previously been recorded from the larvae of *Chironomus* (Léger, 1902) and *Ptychoptera* (Léger and Duboscq, 1909), and the second from *Ephemerella* (Hesse, 1903), all of which are insect larvae living alongside caddis-worms, and probably feeding very similarly. The likelihood of mutual infection with common parasites is, in such cases, very great. I thus feel strengthened in the view, expressed in my last paper, that the spirochaete of trichopterids is identical with those found in *Chironomus* (Léger, 1902) and in *Ptychoptera contaminata*

[1] Through an oversight I gave the specific name of this trichomonad as "*trichopterae*." I take this opportunity of correcting it to "*trichopterorum*."

(Léger and Duboscq, 1909); and I believe that the same spirochaete will be found in other aquatic insect larvae in the future.

Observations such as these lead one to protest against the present tendency to regard each new parasite as peculiar to one host—a tendency which leads to the frequent formation of new species on insufficient grounds, and is surely opposed to the principles of common-sense.

On two or three occasions I saw some small amoebae in the hind-gut of the caddis-worm. The amoebae were very few in number, and I never obtained any satisfactory stained preparations. I shall content myself here with some brief observations made on the living organisms.

MATERIAL AND METHODS.

The caddis-flies and their larvae and pupae were collected in the neighbourhood of Aberdeen at various times from April 1909 till November 1910. Larvae from ponds near Dundee were found to be similarly infected. The infected individuals belonged mainly to the genera *Limnophilus* and *Anabolia*.

The microsporidian occurred in the fat-body of the larva: with this exception, the parasites were all found in the hind-gut along with the trichomonad. I saw no trace of them in the upper regions of the alimentary tract.

I attach prime importance to the study of the living parasites. This can be done either by observing them as they move about within the transparent gut of the young larva, or in teased-out preparations of the gut contents under a waxed-down coverslip.

Examination in soda solution of material that had been fixed in osmic vapour (Schewiakoff's method) was found useful for determining the number of flagella, and their point of origin.

For permanent preparations, fixation with sublimate alcohol (Schaudinn's formula) and staining with Heidenhain's iron-haematoxylin gave the best results in the case of the flagellates. Attempts to stain *Embadomonas* by the modified haematoxylin method used by Rosenbusch for trypanosomes (*Arch. f. Protistenk.* XV. 1909, p. 263) proved unsuccessful.

The microsporidian was first smeared, and then fixed and stained variously; but better results were got by fixing the infected larva whole, and cutting sections of it: portions of these sections were quite thin enough to display the individual spores. Fixation with sublimate

alcohol and staining with Delafield's haematoxylin was found the most satisfactory method. Iron-haematoxylin was also successful; and prolonged staining with Giemsa's stain, followed by rapid dehydration and clearing, was useful.

Description of the Parasites.

(*a*) *Embadomonas agilis*, nov. gen. et nov. sp.

This interesting new flagellate was found in about 60% of the trichopterid larvae examined by me. I never came across it in any of the pupae, but on one occasion I found two individuals in the intestine of an adult fly.

The body of the parasite varies from 8 μ to 14 μ in length, with an average maximum breadth of 4·5 μ. It is of definite slipper shape, with the broadest portion about half-way between the middle and the posterior end; thence it tapers back abruptly into a well-defined point (Pl. III, figs. 1 and 2). The anterior end is rounded, very flexible, and usually bent back at a considerable angle to the long axis of the body, forming, as it were, a heel to the slipper. (See Pl. III, fig. 3.) Very striking is the large cytostome, which extends from about 2 μ from the anterior end to a point almost half-way along the body: its posterior border is often produced into a sort of lip. To carry out the slipper simile, we should compare the cytostome to the opening into which the foot would be thrust.

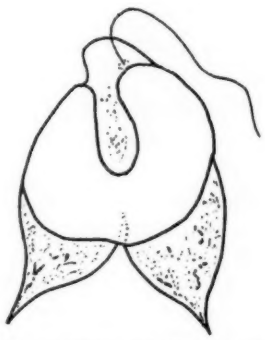
There is only one slender *flagellum*. It arises from a highly refractive point at the anterior margin of the cytostome, and is about three-quarters the length of the body. It describes a wide spiral movement, by means of which the flagellate progresses, rotating with great rapidity round its long axis. The backward inclination of the anterior end of the body leaves the cytostome facing almost directly forward as the animal swims, and bacteria of relatively large size are swept into it in the current produced by the flagellum. It is not unusual to see an *Embadomonas* swimming about with a long bacillus sticking out of its cytostome, which gives it rather a rakish appearance.

In preparations stained with iron-haematoxylin the *nucleus* appears as a diffuse, dark-staining area occupying all the anterior region above the cytostome (Pl. III, figs. 1—3). Well differentiated specimens show a number of dark chromatin masses in a somewhat lighter background. The lateral limits of the nucleus are not definite. Often it is

possible to make out 1—3 dark granules lying on the anterior border of the cytostome. It is very difficult to be certain of the exact point of origin of the flagellum, which is exceedingly slender, and apt to become invisible on differentiation ; but it seems to arise from the most central of these granules.

The *cytoplasm* is often highly vacuolated, and is crowded with masses of bacteria that have been ingested.

Curiously enough, though the flagellate is sometimes pretty abundant, I have only once seen a living individual in process of longitudinal *division*, and I have searched my stained material for division stages in vain. Text-fig. 1 illustrates the dividing flagellate. The cytostome has become widely extended in a lateral direction, while



Text-fig. 1. Drawing of living *Embadomonas agilis*, apparently in process of longitudinal division.

the longitudinal split has already advanced about one-third along the body, starting from the posterior end. I could detect only one flagellum. While I watched, the split advanced a little, and the cytostome showed a tendency to divide in half. At this point, the organism's movements, which had been getting slower and slower, ceased altogether, and it died without completing division. *Embadomonas* is even more sensitive than *Trichomastix*, and seldom survives removal from the host for more than an hour.

I have never seen anything suggesting encystment in this organism.

Systematic position. The name *Embadomonas*—ἐμβάς, a slipper—was suggested by the characteristic shape of the organism. *Embadomonas* does not agree closely with any parasitic flagellate hitherto described, and, in the present unsatisfactory state of the classification,

I cannot define the position that this new genus should occupy. On reading Alexeieff's brief, unillustrated description of *Macrostoma caulleryi* from the tadpole of the frog (*Compt. Rend. Soc. Biol.* (1909), LXVI. p. 199), I was inclined to think that my parasite might be related thereto, even though *Macrostoma* was described as having three flagella and a "vesicular" nucleus. Lately, Wenyon has published a paper (*Parasitology* (1910), III. 2, p. 210) on another species of *Macrostoma*—*M. mesnili*—from human faeces. From this account with its figures, it is abundantly clear to me that *Embadomonas* has very little in common with *Macrostoma*, beyond the possession of a large cytostome.

The new genus may be defined thus: *Slipper-shaped mono-flagellate, with pointed posterior end and blunt, rounded, anterior end, which is bent back at an angle with the long axis of the body. A large cytostome occupies almost all the anterior half of the body. The slender flagellum arises from a basal granule on the anterior margin of the cytostome. The nucleus is a diffuse mass containing several chromatin clumps: it lies in the extreme anterior end, in front of the cytostome.*

(*b*) *Crithidia campanulata*, Léger.

In five of the two hundred larvae examined, the hind-gut contained the adult and encysting stages of a flagellate, which agrees, in all essentials, with *Crithidia campanulata*, Léger. For reasons given above, I do not intend to create a new species, although both previous records of the parasite were drawn from dipterous larvae.

The flagellate was always found in the upper regions of the hind-gut along with the *Trichomastix*. I never saw them in the mid-gut, and my infected material was too limited to allow of complete working out of the life-cycle.

The parasites were mostly in the rounding-off and semi-encysted condition, but a few were still typical *crithidian flagellates* (Pl. III, figs. 4 and 5). Léger and Duboscq (1909) have given a clear and concise description of *Crithidia campanulata* as found in *Ptychoptera*. My parasite does not differ from theirs in any important feature. The larger flagellates are about 12 μ long by 2·5 μ broad: smaller individuals measure only 5 $\mu \times$ ·75 μ. The flagellum projects for some 6—10 μ, and is continued on to the body along the undulating membrane. It takes its origin in a minute basal granule, lying in front of the rod-shaped kineto-nucleus. The kineto-nucleus is pressed up against the anterior

border of the trophonucleus. I saw no division stages among the flagellate individuals, nor any sign of so-called "sexual" forms.

My recent experience of the extraordinary variability of the herpetomonad flagellates and their allies has made me very reluctant to assume from a few samples that my specimen is a true *Crithidia*, and not just a stage in the encystment of a *Herpetomonas*. Particularly is this the case with the flagellates found in the hind-gut, where, in the process of rounding off, a more or less crithidian-like stage is liable to be gone through by *Herpetomonas* itself. I recognize, however, that the present flagellate possesses a slight undulating membrane: according to Patton's definition of the genera this character serves to distinguish *Crithidia* from *Herpetomonas*, even when the other test—*i.e.* the position of the kineto-nucleus relative to the trophonucleus—fails.

The characteristic feature of *C. campanulata* consists in the bell-shaped encysting stages. Pl. III, figs. 9—12 illustrate these, as I found them in the caddis-worms. Léger and Duboscq describe them as attached in masses to the epithelial lining of the gut, with the mouth of each bell directed downwards. I never found the parasite so plentiful as Léger and Duboscq figure it, and the "bells" were more often seen clinging to the bacterial tangles that almost block the intestine of the larva. The "mouth" of the bell seems to arise through the gradual broadening of the anterior end of the flagellate (Pl. III, figs. 6—8) and the withdrawal and dissolution of the attaching flagellum, rather than by the encysting stages coming to lie on their sides, as Léger and Duboscq describe.

Very small, rounded-off individuals measured only 3—4 μ in length, but the final stage of the cyst was not seen.

(c) *Entamoeba*, sp.

In three larvae (*Limnophilus rhombicus*) I noticed a few small amoebae moving about among the bacteria in the hind-gut. Text-figures 2—5 were drawn from the living organisms. I did not succeed in staining them. The average dimensions of the amoebae were about $12\,\mu \times 10\,\mu$. As will be seen from the figures, the form varies from oval to round, one end showing an even outline, while from the other break out several blunt pseudopodia. The endoplasm is very granular, and passes insensibly into the clearer ectoplasm. I saw no contractile vacuole. The nucleus appears as a small, clear, oval or circular area in the posterior portion of the body.

The amoebae moved about rather slowly. On one occasion they showed after some time a tendency to round off and become motionless: I was then able to detect in one or two cases a delicate cyst-wall (Text-fig. 5). Though watched for a long time after that, the amoebae showed no further signs of development. I saw no division stages.

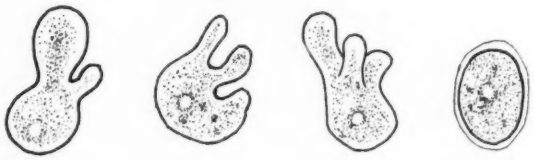


Fig. 2. Fig. 3. Fig. 4. Fig. 5.

Text-figs. 2, 3 and 4. Drawings of living *Entamoeba* from hind-gut of caddis-worm, showing the lobose pseudopodia.
Text-fig. 5. The same, encysted.

Systematic position. The scanty nature of my observations on this form makes it impossible to define a new species. From the rare occurrence of the parasites, and the very slight degree of the infection, I am inclined to suppose that the true host is some other animal, and that their presence in the trichopterid larvae was due to the swallowing of a few chance cysts, which in rare instances have found a suitable field for development. At first I suspected that I might be dealing with *Amoeba chironomi*, Porter, the different form of the pseudopodia being called forth by a slightly different medium. *Amoeba chironomi*, however, is characterized by the possession of a contractile vacuole, an unusual feature in a parasitic amoeba, and one not exhibited by the amoeba in question.

The development of a cyst-wall precludes the possibility of confusion with leucocytes.

(*d*) *Gurleya légeri*, Hesse.

This microsporidian infests the fat-body of the larval caddis-fly. I found it only three times, though I dissected fully two hundred larvae. This is in accordance with the observations of Hesse (1902), who describes it as occurring in the fat-body of only 4 % of the larvae of *Ephemerella ignita*. I think it possible, however, that, as I was primarily interested in the intestinal parasites of my caddis-worms, I may often have overlooked the early stages of the microsporidian in the fat-body. The infected larvae were all found in the late summer of

1910, towards the close of my investigation; the spores were ripe, and the infection so intense that I could not fail to notice it.

The parasitised larva moves about sluggishly, and has a swollen, congested appearance. On opening the body I was at once struck by the curious, chalk-white colour of the fat-body. Examination showed that the whole tissue was replaced by the masses of spores of some microsporidian. These were all at an advanced stage of development: the majority showed four spores within each pansporoblast, but in many cases the outlines of the pansporoblast were lost, and the spores set free.

Figs. 13—14 (Pl. III) illustrate the parasite as I found it. The pansporoblasts are oval to round in form, and measure from $8\,\mu \times 5\,\mu$ to $11\,\mu \times 6\,\mu$. The pear-shaped spores ($4—5\,\mu \times 2{\cdot}5—3\,\mu$) are arranged therein in two superimposed rows: sometimes the narrow ends all point the same way (Text-fig. 6), more often the narrow ends of the upper row lie above the broad ends of the row beneath (Text-figs. 7 and 8). The large pansporoblasts occasionally hold only three spores, and

Fig. 6. Fig. 7. Fig. 8.

Text-figs. 6, 7 and 8. Drawings of the living pansporoblasts of *Gurleya légeri*, to show the arrangements of the spores.

these seem bigger than where there are the usual number. These spores would correspond to Hesse's macrospores, but I do not find the difference in size between the macrospores and microspores so great as he describes it in the parasite from *Ephemerella*. If sulphuric acid be brought in contact with the spores, the filament is shot out to a length of nearly $25\,\mu$.

Systematic position. The genus *Gurleya*, Doflein, is distinguished from other microsporidia of the *Oligosporea* by the possession of tetrasporous pansporoblasts. At present the genus contains three species:

Gurleya tetraspora, Doflein (1898),
G. légeri, Hesse (1903),
and *G. francottei*, Léger and Duboscq (1909).

Of these, the first occurs in the hypodermis of *Daphnia maxima*, and is described as having "oval" spores. Unfortunately, measurements were not taken[1]. *G. francottei*, found in the cells of the intestinal epithelium of *Ptychoptera contaminata*, is a good species with very characteristic crescent-shaped spores.

I see at present no reason for separating the *Gurleya* of trichoptera from *Gurleya légeri*, found in a similar position in *Ephemerella contaminata*, and agreeing with it in all essential points. I regard this as still another instance of the infection by common parasites, to which similar animals on the same feeding-grounds are subject.

From what I have already shown, I think it is clear that trichopterous larvae offer an unusually rich field to the seeker after protist parasites. The following is a list of the parasites hitherto mentioned or described by various authors: with the probable exception of *Gurleya légeri*, they do not seem to be harmful to the host[2].

Class BACTERIA.

Bacillus sp.*
Streptothrix sp.*

Class PROFLAGELLATA, Doflein.

Spirochaeta sp.*

Class FLAGELLATA.

Crithidia campanulata, Léger*.
Embadomonas agilis, nov. gen. et nov. sp.*
Trichomastix trichopterorum, Mackinnon*.

Class RHIZOPODA.

Entamoeba sp.*

Class SPOROZOA.

Sub-Class TELOSPORIDIA.

Ancyrophora uncinata, Léger[3].
Asterophora elegans, Léger.

[1] It would appear to be difficult to distinguish *G. tetraspora* from *G. légeri* on morphological grounds.

[2] Those marked with an asterisk have been recorded by me.

[3] It is interesting to notice that Léger also found this gregarine in the intestine of the aquatic larvae of *Dytiscus* sp. and *Colymbetes* sp.

Discorhynchus truncatus, Léger.
Gregarina mystacidarum, Frantz.
Pileocephalus chinensis, A. Schneider.
P. heerii, Kölliker.

Sub-Class NEOSPORIDIA.
Gurleya légeri, Hesse*.

I take this opportunity of thanking the Carnegie Trust for a grant received during my work.

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EXPLANATION OF PLATE III.

All figures drawn to scale, using Zeiss' achromatic objective 1/12″ or apochromatic 2 mm. homog. immersion, with compensating oculars 8 and 12.

Figures 1—12 stained with iron-haematoxylin, Figs. 13 and 14 with Delafield's haematoxylin.

I. *Embadomonas agilis*, nov. gen. et nov. sp.

Fig. 1. Individual viewed from front, showing cytostome.

Fig. 2. Large individual from same aspect, but with sides of cytostome projecting in lip-like manner.

Fig. 3. *Embadomonas* in side-view, to show slipper-like form.

II. *Crithidia campanulata*, Léger.

Fig. 4. Slender flagellate individual showing narrow undulating membrane, long flagellum, basal granule, kineto-nucleus, and trophonucleus with central karyosome.

Fig. 5. Plump flagellate with well-marked undulating membrane.

Fig. 6. Shortening of flagellum and broadening out of anterior end to form attaching surface of encysting stages.

Fig. 7. Early stage of rounding up.

Fig. 8. Encysting form in which the cytoplasm is full of darkly-staining granules.

Figs. 9—12. Campanulate encysting stages. In fig. 10 the nuclei are dividing.

III. *Gurleya légeri*, Hesse.

Figs. 13 and 14. Typical tetrasporous pansporoblasts. In fig. 14 some of the nuclei are seen in division.

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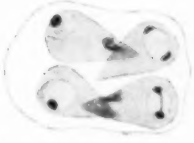

14

D. L. Mackinnon. del.

THE PROCESS OF COPULATION IN *ORNITHODORUS MOUBATA*.

By GEORGE H. F. NUTTALL, F.R.S. and GORDON MERRIMAN.

(*From the Quick Laboratory, University of Cambridge.*)

(With one Text-Figure.)

Although the literature on ticks is very extensive, we have failed to find any satisfactory description therein of the process of copulation. Most authors state that the male mouthparts are introduced into the female genital orifice, or vulva, and that this constitutes copulation, leaving it to the reader's imagination to determine how the seminal secretion gains access to the female generative organs. The mechanism of copulation, in other words, remains to be described, and we propose, in this paper, to give an account of what we have observed in *Ornithodorus moubata*. We shall describe the process in Ixodidae in a later paper, but may state here that, as far as our knowledge goes, it is essentially the same as in *moubata*, that is, impregnation takes place by means of spermatophores.

We have repeatedly observed the process in *moubata* in Cambridge, and the description which follows is based on the study of several pairs in copulation. When a male and female *moubata*, which are ready to copulate, are placed in a dish, the male creeps about upon the female and presently seeks to creep beneath her, usually to one side between the second and third legs. The posterior part of the female's body is now raised, and the male advances so as to bring his body into line with the female's, the ventral surfaces of the pair being apposed. The male clings with his legs to the basal joints of the female's legs, his pair I in

front of pair I of the female, and pair II in front of pair II of the female, and so on. The male all the while feels about with his mouthparts for the female's genital orifice.

The process that follows can, with careful handling of the ticks, be most conveniently observed by placing the pair in a pit made with the end of a pencil in a ball of modelling-wax (plasticine). The ball may measure an inch or more in diameter, and can be rolled about to the desired position, being fixed there at any moment by slight pressure against the glass dish in which it is contained. The female always

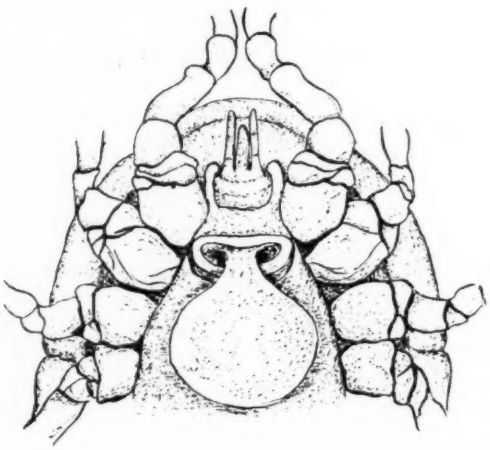

Fig. 1. *Ornithodorus moubata*, ♀. Showing the emptied portion of the spermatophore with the neck-shaped portion attached within the female sexual orifice. Drawn with the aid of a camera lucida (G. M. del.).

seeks to regain her natural position upon a horizontal surface, so it is necessary to immobilize her sufficiently to prevent this without hampering the male. This can be done by modelling the wax about the edge of the pit so that it holds the female in place. Observations on free pairs shows that this manipulation, if done carefully, in no way interferes with the process. All of our observations were made with the aid of a Zeiss binocular microscope which can be inclined at any desired angle.

The male, having found the female's genital orifice by feeling about with his mouthparts, immediately introduces all of these (hypostome,

chelicerae and palps) together into the orifice. The mouthparts are pushed in and withdrawn with a sawing motion, the capitulum being alternately protruded and retracted from his body whilst it is directed backward almost at a right angle to the long axis of the male's body. The mouthparts penetrate more and more deeply but not further than the basal article of the palps. At each movement of partial withdrawal the male mouthparts are tilted forward so that friction against the anterior lip of the vulva takes place. The chelicerae are all the while being actively protruded and retracted within their transparent sheaths, the distance which they travel in their movement to and fro corresponding about to the length of the second palpal article; the chelicerae usually move alternately. Presently the male capitulum is more and more depressed so that the hypostome points slightly backward whilst the dorsal surface of the basis capituli comes to be placed on a line traversing the first pair of coxae about midway along their length when viewed ventrally. (When disturbed at this stage it is seen that the male and female sexual apertures are in apposition.) The ventral surface of the female, together with the thick-lipped vulva, has, up to this, been somewhat prominent, but now the area about the vulva, and especially posterior to it between the first pair of coxae, becomes markedly depressed. At the moment when the female venter becomes depressed the male withdraws his mouthparts completely from the vulva and at the same time separates his body somewhat from that of the female to which it has hitherto been closely applied. The male's capitulum is now directed forward, whilst the palps and chelicerae are working actively, the digits being moved about rapidly. (When the sexes are separated at this stage it is found that the spermatophore is issuing from the male sexual orifice. The spermatophore, viewed as a whole, is flask-shaped, with a long thin neck, which is the first part to issue from the orifice; the tip of the neck of the flask when it issues adheres to the tip of the male hypostome. The spermatophore is ejected rapidly and lies along the venter of the female with the neck directed toward the vulva into which it is immediately inserted by the hypostome of the male. A moment later a large amount of clear-watery fluid emerges from the coxal glands of the male.) When viewed from in front, the ticks not being disturbed, a large amount of clear fluid now floods the space between their venters; the male reintroduces his mouthparts into the vulva, and the ticks again bring their bodies close together. The male mouthparts are worked to and fro very much as they were at first, and,

after two or three minutes, they are finally withdrawn and the male abandons the female.

The ventral surface of the female is found bathed with coxal secretion, the depression about the vulva has disappeared, and the collapsed spermatophore is found attached to the vulva with its neck pushed into the vagina. The whole process of copulation lasts about 15 minutes from the moment that the male inserts his mouthparts for the first time.

The spermatophore, immediately after it has been expelled by the male, appears as a smooth, glistening, very flexible body completely filled with slightly milky contents. (On one occasion when the male was disturbed and the spermatophore was found doubled up on the venter of the female, the spermatophore literally shot out its long thin neck when touched with a fine camel's hair brush in the effort to remove it uninjured.) The end of the neck of the spermatophore appears closed and rounded, whereas the expanded portion of the spermatophore is usually bilobed. When the ticks separate without interference the spermatophore neck appears shorter and the flask collapsed, and somewhat spoon-shaped with the concavity on the side where the male's body had rested upon it whilst *in coitu*. The neck of the spermatophore is firmly attached within the vagina and force is required to remove it; on seizing the spermatophore with a fine forceps the female can be lifted by it without rupturing the structure. On exerting more force the neck of the flask tears loose at the point of entrance into the vagina and the "mouth" of the spermatophore appears spread out and frayed. The full spermatophore is crowded with "prospermiums" and secretion rich in granules; when almost collapsed, a few male elements may still be found therein. The spermatophore, very soon after coitus has taken place, contracts, flattens and hardens, the edges of the "spoon" remaining upturned. We possess several females in our collection which have died or been preserved, with usually one, but at times two or even three empty spermatophores still adhering. The flask-like covering of the spermatophore is exceedingly tough, and tested with 10% caustic potash, it is found to be composed of *chitin*. The freshly expelled spermatophore, with fully extended neck, measures about 3 to 3·5 mm. in length and about 1·7 mm. in width at the widest part. When dried and attached to a female, to whom it may remain attached for some days, or even for weeks, it measures about 1·6 mm. in length.

Although there are several points still to be elucidated with regard to this interesting process, we consider that what we have observed explains in the main how coitus takes place. The introduction of

the male mouthparts necessarily dilates the female sexual orifice and doubtless excites the female. It is possible that a sticky secretion is given off by the male mouthparts which serves to attach the neck of the spermatophore so that its contents are fully voided into the vagina. The male brings his sexual aperture practically into apposition with that of the female, and when she is ready for impregnation, he withdraws his mouthparts and expels the spermatophore which issues with the neck directed forward so that it adheres to the tip of his hypostome and is promptly pushed into the vagina. The male removes his venter from that of the female so as to give space for the spermatophore to pass, and the female venter at the same time becomes concave for the same purpose. The flood of secretion from the male coxal glands serves to prevent the too rapid drying of the chitinous envelope of the spermatophore and keeps it highly flexible until its contents have been discharged —this may be especially necessary in such ticks as *O. moubata* which live in arid climates. The male reintroduces his mouthparts so as to "plant" the neck of the spermatophore in the vagina and he appears to rupture the tip of the spermatophore so that the contents escape into the spermatheca when the bodies of the ticks are brought together again closely and press upon the spermatophore, thus pumping out its contents.

Judging from the fact that we have not as yet observed empty spermatophores adhering to the external genitalia of female ticks belonging to other species, we must conclude that in other cases the spermatophores are introduced *in toto* into the spermatheca. This may be the case in *O. savignyi*, for Christophers (1906, p. 42) states that he found the "spermatozoa" (prospermiums) in this species "included in cyst-like spermatophores" within the spermatheca of the female. He figures two such spermatophores of *savignyi* and also flask-shaped spermatophores in the spermatheca of *Rhipicephalus sanguineus*. Christophers found spermatozoa in the oviducts of *O. savignyi*. Samson (1909, p. 186) has made a suggestive observation on *Ixodes ricinus*, which indicates that copulation takes place in an analogous manner in this species; she merely states, without giving any particulars, that the male impregnates the female by means of spermatophores. In this species, Bertkau (1881, p. 147) and Samson (1909 a, p. 216) have found that the spermatozoa pass up from the spermatheca into the ovaries and there fertilize the eggs. We have also observed them in this situation. Samson (1909 b, p. 495) states that the spermatozoa in *O. moubata* only attain their full development in the ovary.

We have dwelt thus at length upon the process of copulation in *O. moubata*, because until now it has never been understood how the male impregnates the female.

As bearing on the process of copulation in Ixodidae we would add the following remarks:

We have stated elsewhere that the genus *Ixodes* (group Prostriata) is widely separated from the other genera of Ixodidae (group Metastriata) in important points of anatomical structure. In *Ixodes* the anal grooves surround the anus in front, the sexes show marked dimorphism in respect to the structure of the hypostome, and in certain species the sexes are frequently found coupling upon the host. In *Ixodes* the male inserts his mouth-parts into the female sexual orifice as a part of the sexual act. In none of the Metastriata has a similar method of coupling been observed; here the males remain attached for longer periods to the host and they do not appear to wander about upon it as do some species of *Ixodes* whose males infest the host together with the females. In Metastriata the females and males are found attached to the host so that in many cases the venters of the sexes are apposed.

We have discovered that in the Metastriata (including the genera *Haemaphysalis, Dermacentor, Rhipicentor, Rhipicephalus, Boophilus, Margaropus, Hyalomma, Amblyomma* and *Aponomma*) all the males possess a chitinous apron or flap covering the sexual aperture. The apron arises from the integument anteriorly to the sexual aperture and is directed backward over the aperture; it is frequently serrated along its rounded posterior border which is free. The apron can be readily lifted by inserting a fine needle between it and the body surface; it is very thin at the free margin, whereas it grows gradually thicker toward its origin. The apron is absent or rudimentary in the females.

Now we find that in *Ixodes* the males do not show an apron, or it is very rudimentary in character. On the other hand the apron, though very thin, may be very well developed in the females; is best seen in young specimens.

The presence of the apron in the males of Metastriata and its absence in *Ixodes* is very suggestive, and, taken together with the other differences, indicates that copulation may possibly take place in another manner in the Metastriata to what it does in *Ixodes*. The mechanism of copulation in Metastriata still remains to be determined and the significance of the apron requires to be explained.

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ON THE ADAPTATION OF TICKS TO THE HABITS OF THEIR HOSTS[1].

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

(With 26 Text-Figures.)

In the course of the extended investigation upon ticks which we have been conducting for some years, a very large number of specimens have reached us from all parts of the world, thanks to the generous aid which we have received from numerous collectors. In addition to studying this material, the literature on ticks has been sifted for any information which might throw light upon these parasites. A study of the data relating to the structure and habits of ticks and of their host relationships has brought to light certain facts which possess considerable interest and consequently appear to me worth recording in this Journal.

The superfamily *Ixodoidea* is divided into two families, the *Argasidae* and *Ixodidae*, which are distinguished from each other by their external structure as well as by their habits[2].

General considerations regarding the Biology of the Argasidae and Ixodidae.

The *Argasidae* are mostly inhabitants of warm climates. When they occur in colder parts of the world they seek hosts whose habitats afford them protection, and insure them possibly a certain degree of

[1] Read before the *Cambridge Philosophical Society* Meeting, 20 February, 1911.

[2] The main structural differences are described by Nuttall and Warburton (1908) in *Ticks: a Monograph of the Ixodoidea*, by Nuttall, Warburton, Cooper and Robinson, Part I, p. 1.

warmth from proximity to animals upon which they feed. Thus, *Argas reflexus* and *Argas vespertilionis*, which occur in colder climates, are parasitic on pigeons and bats respectively, and they obtain shelter in pigeon coops and the retreats of bats.

Like all other ticks, they pass through larval and nymphal stages before attaining maturity. In some Argasidae the *larvae* suck blood (as in Ixodidae); in others they do not. Where the larvae suck blood they remain attached to the host for 5—10 days or more (as in Ixodidae). There are two or more *nymphal stages* in Argasidae (one only in Ixodidae), and the nymphs, with one exception, *Ornithodorus megnini*, are rapid feeders, that is, they usually feed on blood to repletion within 20—30 minutes (the nymphs of Ixodidae take several days to become gorged). The *adults* are not only rapid feeders, but they may feed repeatedly (not so in ♀ Ixodidae, though possibly in ♂s).

The nymphs[1] and adults of Argasidae are *rarely* carried away from the habitats by their hosts because they feed rapidly. They are mostly nocturnal feeders, attacking the host when the latter is sleeping or resting. All species of Argasidae would appear to shun the light, especially when waiting for a host. When hungry they may leave their hiding places during the daytime if they become aware of the proximity of a host. The Argasidae infest the habitat of the host.

The nymphs and adults of *Ixodidae* behave in a very different manner. The nymphs attach themselves to the host, and are carried about for several days, and possibly longer. This likewise holds for the adults of both sexes in a large number of species. The duration of their parasitism upon the host may be much prolonged, especially when the host is a cold-blooded animal; thus, species of *Amblyomma*, *Aponomma* and *Hyalomma*, occurring on Reptilia, may remain attached to the host for many months. On the other hand, we shall see that there are species of *Ixodes* in which the males are unknown or in which they are only recorded as having been found in the habitats of their hosts.

In the Argasidae the females lay eggs in batches after successive feeds, and the total number of eggs laid by a female is comparatively small, say 200. In the Ixodidae, the eggs may number one or more thousands. The explanation of this difference in the number of eggs laid appears obvious when we consider the life habits of the two families. The Argasidae are essentially *ticks of the habitat* in their nymphal[2] and adult stages; the females lay their eggs in and about

[1] *Ornithodorus megnini* excepted.

[2] *O. megnini* excepted.

their host's retreat and naturally the offspring have a good chance of finding the host when their turn for feeding arrives.

With the Ixodidae the case is different: the fertilized and replete females must frequently drop from the host when it is on its wanderings, and the offspring may be left stranded in places where they may never find a host. The loss of life in nature must be enormous in the majority of species, for in most species the larva, nymph and adult must each seek a host. The loss of life should be less in those requiring but one host, as in *Boophilus*, where the tick attacks the host in the larval stage and undergoes its metamorphosis up to the adult stage upon a single animal. The greatest loss of life must of necessity occur in such species as are parasitic upon hosts having no fixed habitat. In other cases, owing to the fact that the host returns to a burrow, nest, or the like, it is probable that there may be less loss of life than in the case where an animal with wandering habits is chosen as a host. It would be interesting to make some numerical determinations of the progeny, especially of various species of Ixodidae, with this point of view in mind.

The large progeny of the Ixodidae secures their survival in nature in the face of the many difficulties the ticks must encounter in finding their hosts. In the Argasidae these difficulties are much smaller and the progeny is proportionately less numerous.

Argasidae.

In the following table I have included all the Argasidae: *Argas* (*A.*) and *Ornithodorus* (*O.*) of which we know the host relationships. The list includes 12 species and 1 sub-species:

Argasidae	Hosts and habitat etc. of Tick	Geographical distribution (as far as known)
A. persicus ...	Birds: fowls, ducks, geese, turkeys, ostriches, quail, wild doves, canaries. Infests chicken coops, etc. Man: prevalent in Persia where it infests native dwellings. It also occurs in houses in Egypt.	Africa, Asia, Australia, N. & S. America.
A. reflexus ...	Birds: pigeons, fowls. Man: occasionally entering dwellings from pigeon coops, or attacking persons having to do with pigeons.	Europe, N. Africa.
A. vespertilionis	Bats: several species[1]; adults found in the abodes of bats.	Europe, N. Africa.

[1] See *Ticks*, Part I, p. 39, where the bats are listed. A variety infests penguins at Queenstown, Cape Colony.

Argasidae	Hosts and habitat etc. of Tick	Geographical distribution (as far as known)
A. brumpti ...	Porcupine: found infesting dusty burrows of *Hystrix* sp. in Africa.	Africa.
	Man: attacks man when sleeping on the ground; hides in dust during the day-time.	
O. savignyi ...	Man, camel, horse: attacks them in their resting places, hides in dust or sand, etc.	Africa, India.
O. moubata ...	Man: infests native dwellings and resting places along caravan routes.	Africa.
	Domesticated animals: dogs, pigs kept in styes, goats, sheep.	
O. turicata ...	Man: at times infesting native dwellings; attacks at night.	N. & S. America.
	Domesticated animals: pigs in styes, cattle, llama, horse.	
	Wild animals: infesting burrows of tortoise and gopher.	
O. talaje ...	Man: infesting native houses; attacking at night.	N. & S. America.
O. talaje var. *capensis*	Birds: found in penguin's and other nests.	Islands of Cape Colony, St Paul's Isl., Siren Isl.
O. pavimentosus	Man: resting places infested.	Africa.
O. tholozani ...	Fowls and camels: infests chicken coops.	Persia.
O. lahorensis ...	Sheep: no particulars as to manner in which sheep are kept.	India.
O. megnini ...	Man and domesticated animals: horse, ass, ox, are all the hosts recorded; occurring chiefly in the ears.	N. America.

Of the foregoing species the life-histories of only five are known (*A. persicus*, *A. reflexus*, *O. moubata*, *O. savignyi*, and *O. megnini*), the ticks having been raised experimentally. The various stages of *A. vespertilionis* are known, and nymphal or adult stages (or both) of some of the other species are more or less known to science. In *A. persicus*, *A. reflexus*, *A. vespertilionis* and *O. megnini* the larvae attack the host. In *O. moubata* and *O. savignyi*, in which the eggs are correspondingly large, the larva is formed within the egg, but it is incapable of attacking a host; the larva in both of these species is inactive, and very soon after the egg-shell splits and whilst it remains in (*moubata*) or near (*savignyi*) the egg-shell it rapidly undergoes metamorphosis and emerges from the larval skin as a nymph. It is as a nymph that both the latter species first attack the host.

	remain attached to the host for
The larvae of	
A. persicus	5 and 10 days in warm and cool weather respectively (Nuttall).
A. reflexus	7 days (Brumpt, 1910, p. 528).
A. vespertilionis	Some days at least (Nuttall)[1].
O. megnini	5 days (but they stay on as nymphs upon the host).
The first stage nymphs of	
A. persicus	1½—2 hours (Nuttall).
O. moubata	10 minutes—1 hour (Nuttall).
O. megnini	35—98 days, or more (Hooker); abandons the host as a late stage nymph and does not attack a host as an adult.
The latter stage nymphs and adults of	
A. persicus	5 minutes—2 hours (Nuttall). (Usually less than ½ hour.)
A. reflexus	20—27 minutes (Alt, Boschulte).
O. moubata	20 minutes—2 hours (Nuttall).
O. coriaceus	45 minutes—1⅔ hours (Nuttall).
O. turicata	Feed more rapidly than the last (Nuttall).
O. tholozani	30 minutes (Mégnin).
O. lahorensis	25 minutes—2⅓ hours (Nuttall).
O. savignyi	Feed like *O. moubata* (Nuttall).

We may divide the ticks above enumerated into three classes, according to their biology:

Group 1. *A. persicus, reflexus,* and *vespertilionis.*
„ 2. *O. moubata* and *savignyi.*
„ 3. *O. megnini.*

Group 1. *A. persicus, reflexus and vespertilionis.*

In this group the larvae are parasitic upon the host for, say, 5 to 10 days. In *persicus,* and this probably holds for the two other species, the nymphs feed about as rapidly as the adults, *i.e.* in less than 2 hours; at times in 20—30 minutes. This explains why these stages are infrequently found upon the host; they are rapid feeders and quickly abandon the host when replete. *Argas persicus* and *reflexus* are essentially parasitic on birds. There is no trustworthy record[2] of the larval stages having been found on man or upon other mammalian hosts. Without denying the possibility of their attacking mammals, it

[1] Although I have examined many bats, I have never found *A. vespertilionis* adults and nymphs upon them. On the other hand, larvae, in various stages of repletion, were not infrequently encountered. I conclude from this that the larval stage must remain attached to the host for some days at least. Doubtless, the duration of parasitism is influenced by the surrounding temperature.

[2] It is reported that Starcovici once found *reflexus* larvae on the horse in Roumania (*vide Ticks,* Part I, p. 27), but I feel warranted in doubting the statement.

appears reasonable to suppose that when they infest human dwellings it is because of birds being harboured there. The birds on which the ticks have been found are nearly all domesticated. The natural hosts of *persicus* and *reflexus* are commonly fowls and pigeons which doubtless have served to spread the tick amongst other birds in their vicinity. The thin skin of the bird seems peculiarly adapted for the successful attack of the small larval tick, and the nesting or roosting habit of the birds permits the ticks to drop off in situations adapted to the feeding and other life habits of the nymphs and adults. Both species in their mature stages are essentially night feeders, that is, they feed when the birds are in the dark and asleep, and the ticks retreat into crevices and dark places during the day-time. Where the ticks attack man they do so at night-time. I assume that *O. talaje* var. *capensis*, which has been found in penguin's and other birds' nests, will be found to have a similar life-history to the above; the larvae should be found on the birds in localities infested by the tick.

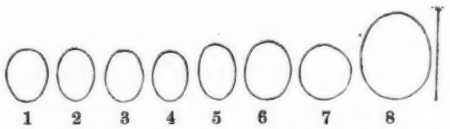
Argas vespertilionis has never been found on other animals than bats[1]. Here we have a host which retreats to dark places during the day-time. The retreats of bats (hollow trees, beneath roofs of houses, in caves and grottoes) are more or less permanent dwellings where the ticks find shelter very much as do the Argasids above mentioned which attack birds. It is clear that to secure the adult stages of this tick the habitats of bats are the places in which to search for them.

Group 2. *O. moubata and O. savignyi.*

In this group the larvae are inactive, and the first stage nymph attacks the host. This nymph feeds as rapidly (in the case of *moubata*) as does the adult, *i.e.* in 10 minutes to one hour. *Ornithodorus moubata* and *savignyi* seem to be peculiarly adapted to their hosts and the habits of their hosts. All of the latter, *i.e.* man and the domesticated animals possess thick skins. Man appears to be the chief host, at any rate of *moubata*. The hairless human skin, coupled with man's ability to remove his ectoparasites, renders prolonged parasitism difficult. Man herds his animals into a limited space in immediate proximity to his dwellings or resting places along routes of travel. Both species live in hot dry climates where there is a largely unclothed human population. The ground is for long periods of the year covered deeply with a layer of dry dust or sand both in and out of dwellings.

[1] See footnote p. 48.

Both species, if they had active larvae, would labour under a considerable disadvantage under these conditions and an enormous number of larvae would be lost in the dust or sand, and would die from desiccation. It may be noted that the females in captivity burrow and lay their eggs above them on or near the surface of sand. To overcome these conditions they must be provided with a thick integument to protect them against desiccation; they must possess mouth-parts capable of penetrating a thick skin and of drawing blood rapidly; an increase in size and



Figs. 1—8. Eggs of Ixodoidea showing their relative size in some species belonging to different genera. Outlines drawn with a camera-lucida and reduced. The scale to the right = 1 mm. (Original. G.M. del.):

1. *Ixodes hexagonus* 575 × 450 μ.
2. *Haemaphysalis punctata* 570 × 380 μ.
3. *Hyalomma aegyptium* 550 × 425 μ.
4. *Rhipicephalus evertsi* 550 × 400 μ.
5. *Boophilus decoloratus* 625 × 400 μ.
6. *Amblyomma hebraeum* 625 × 525 μ.
7. *Argas persicus* 625 × 575 μ.
8. *Ornithodorus moubata* 950 × 750 μ.

strength will render them more capable of dealing with the dust problem. The larval stage is, therefore, inactive. The females lay larger eggs (and fewer) than any other ticks, so that they contain enough reserve upon which the nymphs may develop. The first stage nymphs are rapid feeders, and very resistant to drought and prolonged starvation, although somewhat less so than the later stages.

Group 3. *O. megnini.*

Ornithodorus megnini is quite aberrant in its habits, and appears highly specialized in its adaptation to a life of parasitism. As in Group 1, the larva attacks the host and remains attached thereto for about five days, by which time it is replete. It moults *in situ*, and re-attaches itself to the host for 35—98 days, or more, slowly growing in size and doubtless moulting as do the nymphs of other Argasidae. The larvae and nymphs are especially prone to attack the ears of their hosts. When replete, the nymphs abandon the host, crawl up several feet on posts, trees, or the like, and hide in chinks and crevices. After about seven days, in summer, the adults emerge, but they do not seek

a host. During their long period of parasitism as nymphs they have doubtless undergone internal changes leading to maturity, otherwise they would not emerge so rapidly as adults from the nymphal skin. The adults, without further feeding, mate; the females oviposit in the chinks and crevices, and the larvae which emerge from the eggs await the coming of a host. Hooker, to whom we are indebted for most of what is known of this curious life-history, very naturally regards the habits of this tick as correlated with its peculiar form of parasitism in the ears of its hosts. He does not, however, discuss the subject.

Animals rubbing their ears or heads against infested posts or trees ("rubbing places") would necessarily pick up the waiting larvae. I would add that the structure of the mouth-parts, and especially the presence of an unarmed hypostome in the adults, indicate that they can scarcely be blood-suckers. It would be a matter of considerable interest, it seems to me, to examine the internal anatomy of the adults of this species to see in how far it is modified, especially with regard to the pharyngeal pump, salivary glands and digestive organs.

The life-history of *O. megnini* is that of an animal highly specialized to a life of parasitism chiefly in the ears of its hosts. The larvae issue from eggs laid on rubbing posts or trees, and the like, some feet above the surface of the ground, so that they may readily gain access to the heads of their hosts. The larvae enter the ear unperceived. It would be a matter of increasing difficulty for succeeding stages to "cast anchor in such a small harbour" a second time for, apart from their increasing size, which would militate against their re-entering the ear, the number of ticks of later stages which survive even from a large batch of larvae (in all species of ticks) is very small and grows progressively smaller toward the period when maturity is reached. The tick, having entered the ear where it is safe for an unlimited time, takes advantage of the protection afforded and only leaves it once when it abandons it as a large nymph almost ready to cast its skin and emerge as an adult. The period of parasitism as a larva is normal, but that of the nymphal stages inordinately prolonged so that the tick only needs to go in and out once through the small aperture of the ear. It is owing to the choice of the ear for its place of feeding upon the host that the life-history of this tick has become so peculiarly modified. Its spiny integument, to whom it owes its name of "spinose ear tick," may be of use in connexion with its parasitism in the ear in lessening its chances of adhering with a large body-surface to the cerumen within the ear.

Of the other species of Argasidae which have been enumerated in the list on pp. 48, 49, too little is known to permit of much comment. It would seem to me as if *A. brumpti*, *O. turicata* and *O. pavimentosus* may prove, on investigation, to have a life-history similar to that of *O. moubata* and *O. savignyi*; *O. talaje* and *O. tholozani* may possess life-histories like *A. persicus*, and this appears highly probable for *O. talaje* var. *capensis*. It is to be hoped that the life-history of *O. lahorensis* will be worked out in India. We are studying *O. turicata* and *O. talaje* var. *capensis*.

Note on the structure of the Hypostomes in Argasidae.

The dentition of the hypostome is in accord with what I have stated regarding the feeding habits of the Argasidae. This is illustrated by typical examples in the accompanying figures. Thus, in *A. persicus*, *reflexus* and *vespertilionis*, where the larvae attach themselves to the host for some days, or longer, the larval hypostome is

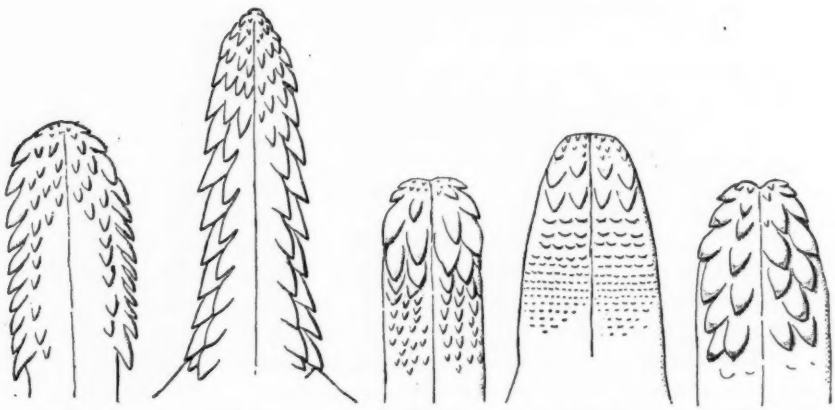


Fig. 9[1]. Fig. 10. Fig. 11. Fig. 12. Fig. 13.

Fig. 9. *A. persicus*, larva (similar in larva of *A. reflexus*)
Fig. 10. *A. vespertilionis*, larva.
Fig. 11. *A. persicus*, adult.
Fig. 12. *A. reflexus*, adult.
Fig. 13. *A. vespertilionis*, nymph.

[1] Figs. 9—14 are from Neumann, being reproduced (as are also Figs. 15, 16) from *Ticks*, Part I.

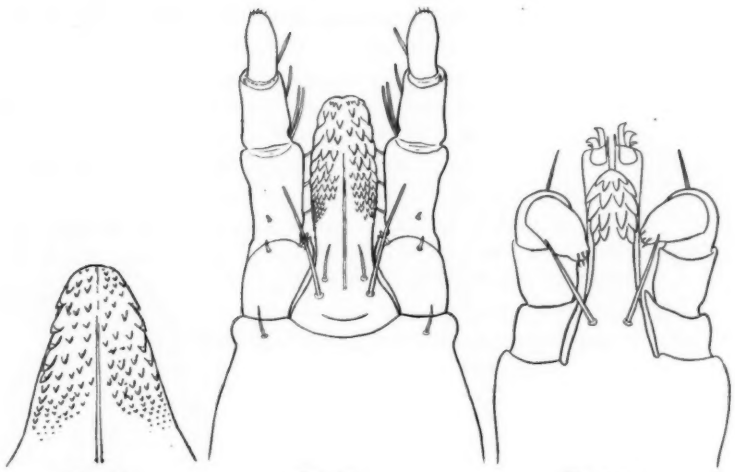


Fig. 14. Fig. 15. Fig. 16.

Fig. 14. *O. savignyi*, adult. Fig. 15. *O. moubata*, adult.
Fig. 16. *O. moubata*, 1st stage nymph.

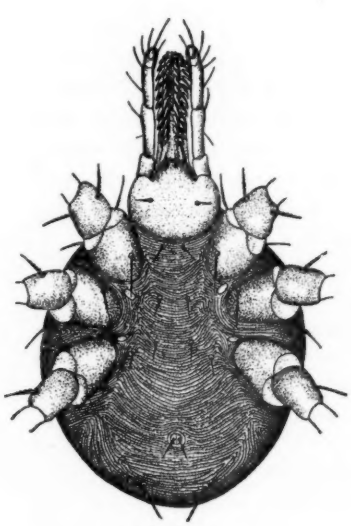


Fig. 17[1].

Fig. 17. *O. megnini*, larva.

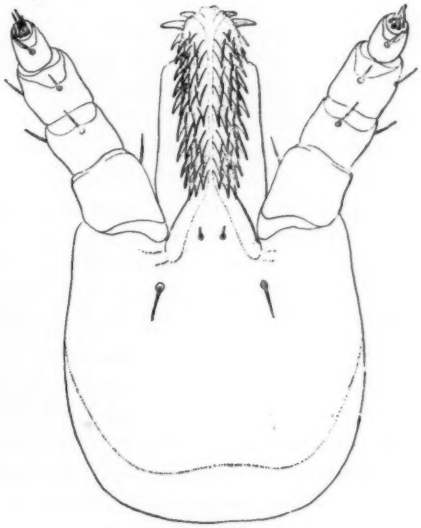


Fig. 18[1].

Fig. 18. *O. megnini*, nymph.

[1] Reproduced from Salmon and Stiles, 1901, figs. 99 and 102 (*17th Ann. Rep., B. A. I., U. S. Dept. Agricult.* and from *Ticks*, Part I.

armed with long pointed teeth running down to its base (Figs. 9, 10). The adults or nymphs of these species (Figs. 11, 12, 13) show a considerable reduction in the armature of their hypostome. In *O. savignyi* and *O. moubata*, where the larvae are inactive and the nymphs and adults are both rapid feeders, the dentition of the hypostome is reduced (Figs. 14, 15, 16). In *O. megnini* the larval hypostome (Fig. 17) is powerfully armed, and the same is the case with that of the nymph (Fig. 18), which we have seen remains attached for a long time to the host. The hypostome of the adults, which do not suck blood, is *unarmed*, and the capitulum, as a whole, is quite exceptionally small. The figures are reproduced from *Ticks*, Part I, without regard to the relative magnifications employed.

Ixodes.

When we come to consider the biology of *Ixodes*, which are clearly marked off from other Ixodidae structurally, there are two points which are very striking in respect to the adult stages: (1) the considerable number of species in which the females only are known, and (2) the fact that the males of some species are frequently found in copula on their hosts (in some species the sexes remain in copula even when they are dropped into spirit). In no other genus of *Ixodoidea* is this the case. On the other hand, in those species in which both sexes are known, some are found in copula upon the host, whilst others are not. I have sought a reason for these peculiarities in *Ixodes*, and believe I have found an explanation of these differences. I shall begin by discussing the host relationships in *Ixodes*.

Of the 51 species listed we have no data regarding the hosts of nine, namely, *nigricans, fossulatus, acutitarsus, gigas* (♂), *japonensis, percavatus, cordifer* (♂), *tasmani* and *coxaefurcatus* (♂). We nevertheless include two of these (*percavatus* and *tasmani*) in the subjoined lists because the presumption appears justified that they came from birds: *percavatus* came from isolated islands in the Pacific inhabited by birds, and we possess a variety of this species, taken from a puffin; *tasmani* was collected on the Island of St Pierre, and by the celebrated ornithologist, Jules Verreaux, in Tasmania. We therefore are able to list 44 species in relation to their hosts, there being but two of these (*percavatus* and *tasmani*) with regard to which the evidence is not quite satisfactory.

In the appended lists I have grouped the various species of *Ixodes* as follows:

I. Species of which both the ♂ and ♀ are recorded as occurring upon the host.

(*a*) Species in which the sexes have been found in copula upon the host.

(*b*) Species in which the sexes have been found near together upon the host.

II. Species of which the females only have been found upon the host.

III. Species of which the females only are known.

The immature stages of these ticks are left out of consideration in these lists. Alongside the name of the hosts are placed certain signs:

+ denotes a host possessing wandering habits.

0 denotes a host with fixed habitat for more or less prolonged periods; the habitats being burrows, nests, caves, hollow trees, etc.

± is used in the case of *Canis familiaris*, as in some places it may be fittingly described as a wandering host, in others as a host with fixed habitat (the kennel).

A consideration of the data tabulated in the following pages brings out certain striking facts. The ticks included under Group 1 (*a*) are, in most cases, encountered upon (+) *wandering hosts*. In the case of *ricinus*, our records show that it has been found 63 times on wandering hosts and only 10 times on hosts which burrow. Being a very prevalent and hardy species, it is natural that it should occasionally occur on a variety of hosts. The tick may at times infest dog kennels, thus taking advantage of the fixed habitat of the host. The three varieties of *ricinus* (var. *scapularis*, *ovatus* and *californicus*) appear to follow the same rule, and in the case of *pilosus* we appear to have a striking example of a tick occurring upon wandering hosts.

Group 1 (*b*). The species included in this group occur, in some cases, on wandering hosts, in others not. Thus, *angustus* and *tenuirostris*, according to my notes, occur solely on nesting and burrowing animals. In *angustus* we have a form in which the males occur very rarely upon the host, and in which the male hypostome[1] is poorly armed; *angustus* may, therefore, be regarded as an intermediate form in respect to its adaptation. In *tenuirostris* we have a tick which, as far as we know, only

[1] The variations in this structure in male *Ixodes* will be discussed presently.

I. *Species of Ixodes of which both ♂ and ♀ are recorded as occurring upon the host.*

(*a*) *Species in which the sexes occur in copula upon the host.*

Species	Host's habits	Host	Number of times found thereon		Geographical distribution of tick
I. ricinus	+	Bos taurus	13		Europe, N. Africa,
	+	Ovis aries	13		W. Asia, W. America.
	±	Canis familiaris	13		
	+	Cervus elaphus	5		
	+	,, capreolus ...	4		
	+	,, dama	1	63	
	+	Capra hircus	2		
	+	Equus caballus	6		
		Homo sapiens	2		
	+	Felis concolor	1		
	+	Genetta sp.	1		
	+	Lepus europaeus... ...	2		
	0	Erinaceus europaeus ...	2		
	0	Mustela putorius ...	1		
	0	,, erminea... ...	1		
	0	Meles taxus	1		
	0	Mus decumanus	1	10	
	0	"Mouse"	1		
	0	Myoxus sp.	1		
	0	Talpa europaea	1		
	0	Ground squirrel	1		
		Larvae and nymphs on Lizards (10) and birds (5) of different species.			
I. ricinus var. *scapularis* (♂ ♀ known)	+	Cattle			N. America, Costa Rica.
	+	Deer			
	±	Dog			
		Man			
I. ricinus var. *ovatus* (only 2 ♀ known)	+	Horse			Japan.
	±	Dog			
I. ricinus var. *californicus*	+	Black-tail deer			N. America.
	0	Grey fox			
I. pilosus	+	Capra hircus	3		S. Africa.
	+	Bos taurus	2		
	+	Equus caballus	3		
	+	Sus scrofa domestica ...	2		
	+	Ovis aries	1		
	+	Duikerbok	1		
	+	Tragelaphus sylvaticus ...	1		
	±	Canis familiaris	1		

Species	Host's habits	Host	Number of times found thereon	Geographical distribution of tick
I. pilosus	+	Felis domesticus	1	S. Africa.
	+	Viverra civetta	1	
	+	Felis pardus	1	
		Homo sapiens	1	
I. pilosus var. *howardi* (♂ ♀ known)	+	Cat		S. Africa.
	0	Erinaceus frontalis		
	0	Rhinolophus sp.		
I. boliviensis ...	?	Icticyon (Speothos) venaticus (Canidae)	1	Bolivia.
I. schillingsi ...	+	Colobus caudatus (monkey)	2	E. Africa.
I. rasus	+	Bos taurus		E. & W. Africa.
	+	Felis pardus		
	±	Canis familiaris		
	0	Herpestes ichneumon		
	0	Hyrax sp.		
		Homo sapiens		
I. ugandanus ...	+	Ovis aries		E. & W. Africa.
	0	Aulacodus sp.		
		"Large rodent" (? Aulacodus)		
I. ugandanus var. *djaronensis* (♂ ♀ known)	?	Genetta suahelica ...		E. Africa.
	0	Procavia brucei		

(*b*) *Species in which the sexes have been found together upon the host.*

I. angustus ...	0	Sciurus hudsonius douglasi		N. America.
	0	Tamias townsendi (Sciuridae)		
	?	Lepus dalli		
	0	Neotoma occidentalis (Muridae)		
	0	"Mouse"		
I. tenuirostris ...	0	Evotomys glareolus ...	3	Europe.
	0	Arvicola amphibius ...	2	
	0	,, pratensis ...	1	
	0	Microtus agrestis ...	2	
	0	,, arvalis	1	
	0	Sorex vulgaris	1	
	0	,, minutus	1	
	0	Mus miniatus	1	
I. minor	0	Hesperomys sp. (Muridae)	1	Guatemala.
I. cavipalpus ...	+	Cynocephalus babuin ...	1	Africa (Rhodesia & Angola).
		Homo sapiens	1	
I. rubicundus ...	+	Ovis aries	1	S. Africa.
	+	Capra hircus	1	

Species	Host's habits	Host	Number of times found thereon	Geographical distribution of tick
I. rubicundus var. *limbatus* (♀ only known)	+	Sheep	1	C. Africa (Congo).
	+	Goats		
I. holocyclus ...	+	Bos taurus		Australia, India.
	+	Macropus sp.		
	±	Canis familiaris		
	0	Sciurus variabilis		
	0	Phascogale penicillata (Marsupial tree shrew)		
I. loricatus ...	+	Ateles melanochoerus		S. America.
	0	Didelphys aurita		
	0	,, opossum		
	0	,, sp.		
	0	Microdidelphys sorex (o & L[1].)		

occurs as a parasite on small mammals which burrow; these burrows are, however, near the surface of the ground and *liable to be destroyed* or flooded, consequently, for the maintenance of the species, it is essential that both sexes should be carried about upon the host as in the case of ticks occurring on hosts with wandering habits. The other species follow the same general lines as 1 (*a*) with regard to their host relationships; the majority of the hosts have wandering habits. In *minor* (only found once) the sexes may occur together on the host for the reasons stated above for *tenuirostris*; *loricatus* appears to be an exception.

If the reader will run his eye down the column of + and 0 signs in the lists I (pp. 58—59) and II (pp. 61—62) of the hosts of the different species of *Ixodes*, he cannot but be struck by the differences. The hosts enumerated in II, under "Species of which the females only have been found upon the host, the males being known," are nearly all animals which burrow or nest. Thus, we record the finding of *hexagonus* no less than 40 times on such hosts, once each on three wandering hosts (if we include man), and three times on the dog. The list of hosts of *hexagonus* var. *cookei* (taken from Banks) falls in with that of *hexagonus*. In the case of *canisuga*, the dog is the most prominent host, simply because nearly all of our specimens from the dog came from the north of England where the shepherd dogs are largely confined in kennels. The dog in these regions is a host with a fixed habitat, as with most of the other hosts; in only two instances out of 23 has this species been recovered from a wandering host.

[1] o and L signify that nymphal and larval stages only were found.

II. *Species of which the females only have been found upon the host, the males being known.*

Species	Host's habits	Host	Number of times found thereon	Geographical distribution of tick
I. hexagonus ...	0	Erinaceus europaeus ...	9	Europe, N. Africa, N. & S. America.
	0	Mustela erminea	8	
	0	,, vulgaris	7	
	0	,, putorius	6	
	0	,, furo	2	
	0	Lutra vulgaris	1	
	0	Meles taxus	1	
	0	Lepus cuniculus	1	
	0	Myopotamus coypu ...	1	
	0	Sciurus sp.	1	
	0	Canis vulpes	3	
	±	Canis familiaris	3	
	+	Ovis aries	1	
	+	Sus scrofa	1	
		Homo sapiens	1	
I. hexagonus var. *cookei* (♂ ♀ known)	0	Lutra		N. America.
	0	Mustela vison		
	0	Spermophilus		
	0	Weasel		
	0	Porcupine		
	0	Marmot		
	0	Pocket gopher		
	±	Dog		
	+	Cat		
	+	Sheep		
I. canisuga ...	±	Canis familiaris	12	Europe, N. America.
	0	,, vulpes	2	
	0	Cotile riparia (bank-swallow)	3	
	0	Mustela furo	1	
	0	Meles taxus	1	
	0	Sciurus	1	
	0	Talpa europaea (larva) ...	1	
	+	Equus caballus	1	
	+	Ovis aries	1	
I. putus. On marine birds only (♂ only found in nests or beneath rocks and soil near nests).				
	0	Phalacrocorax verrucosus ...		Europe, N. & S. America, Asia, Australasia.
	0	Pygosceles taeniatus		
	0	Spheniscus magellanicus		
	0	Fratercula arctica ...	3	
	0	Penguin sp.	2	
	0	Uria troile	1	
	0	Sea-gull sp.	1	
	0	Sula bassana	1	
	0	Fulmarus glacialis ...	1	
	0	Cormorant sp.	1	
	+	Man (occasionally, see text, p. 62)		

Species	Host's habits	Host	Number of times found thereon	Geographical distribution of tick
I. vespertilionis.	Bats only (♂ found in caves etc. inhabited by bats).			
	0	Plecotus auritus		Europe, Africa, Australia.
	0	Rhinolophus hipposideros		
	0	„ ferrum equinum		
	0	„ euryale		
	0	„ hippocrepis		
	0	„ blasius.		
	0	„ clivosus		
	0	Vesperugo pipistrellus		
	0	„ tricolor.		

Ixodes putus and *vespertilionis* are solely parasites of birds and bats, although *putus* may occasionally attack man when he approaches the infested birds' nests.

With the exception of *I. hexagonus*[1], including var. *cookei*, of which we have no particulars, *the males of these species have never, apparently, been captured upon the host.* On the other hand, the males of the other species have been found in the habitats of their hosts: *canisuga* in the nests of sand-martins (bank-swallows) and in a dog kennel, *vespertilionis* in caves inhabited by bats, *putus* in and about the nests of marine birds. In such localities the males would have ample opportunities of feeding upon their hosts for brief periods after the manner of male Argasidae, assuming that they do feed. It is conceivable, however, that such habitat-infesting males may not be blood-suckers at all. We have seen that *Ornithodorus megnini* ♂ does not feed, and that it nevertheless fertilizes the female. The same may hold for certain Ixodidae ♂s. The matter requires further investigation. The sexes of *canisuga* and *putus* have often been observed in copula apart from the host, and it appears probable that this is the usual occurrence in nature.

List III (p. 63) is likewise very striking. It includes all the species (26) of which the males are still unknown. With the exception of three species (*bicornis, stilesi* and *australiensis*), all of the females have been found solely on birds, burrowing or nesting hosts, and bats. Of *I. bicornis* only three females are known; they were found on as many hosts. *Ixodes stilesi* has only been found once, and all the specimens (14 females) were found upon a single host. *Ixodes australiensis* was found once on a dog (±), and once on the marsupial rat-kangaroo (0). It is true that many of the species are represented only by one or two females, or by few specimens, and consequently there may be an error in certain cases in attaching much importance to the character of the type host—nevertheless, there is a very remarkable co-relation between the absence of males on the host and the life habits of the host.

[1] See Note on p. 67.

III. *Species of which the females only are known*[1].

Species	Host's habits	Host	Geographical distribution of tick
I. acuminatus	0	Mus agrarius (only 2 ♀ s known) ...	Italy.
I. dentatus	0	Rabbit (1 ♀ known)	N. America.
I. diversifossus	0	Procyon lotor (2 ♀ s recorded) ...	N. America.
I. fuscipes	0	Dasyprocta aguti (? Felis pardalis) ...	C. & S. America.
I. sculptus	0	"Rock squirrel"	N. America.
I. spinicoxalis	0	Mustela flavigula (4 ♀ known) ...	Sumatra.
I. marxi	0	Red squirrel and fox (few ♀ s known)...	N. America.
I. rubidus	0	Bassaris astuta (1 ♀ known) ...	Mexico.
I. nitens	0	Mus macleari (2 ♀ s known)	Christmas Island, Pacific Ocean.
I. bicornis	+	Felis concolor, F. onca, Homo sapiens (may = diversifossus, only 3 ♂ s known)	Mexico.
I. auritulus	0	Trupialis militaris and another bird, sp.?	S. America.
I. brunneus	0	Birds: many species	Europe, N. America.
I. caledonicus	0	Domestic pigeons	Scotland.
I. simplex	0	Rhinolophus ferrum equinum, Vespertilio	China.
I. texanus	0	Grey squirrel, Procyon lotor (few ♀ s known)	N. America.
I. stilesi	+	Padua humilis (Cervidae); (14 ♀ s found once)	Chili.
I. neumanni	0	Marine birds (10 ♀ s known)... ...	New Zealand.
I. percavatus	0	Marine birds (presumably)	Tristan d'Acunha Island.
I. percavatus var. *rothschildi*	0	Marine bird (puffin)	No locality given.
I. lunatus	0	Hallomys audeberti (1 ♀ known) ...	Madagascar.
I. ornithorhynchi	0	Ornithorhynchus (found 5 times) ...	Tasmania.
I. tasmani	0	Probably birds (Verreaux coll.) ...	,,
I. fecialis	0	Dasyurus geoffroyi (1 ♀ known) ...	Australia.
I. fecialis var. *aegrifossus*	0	Opossum (3 ♀ s); Parameles obesula (1 ♀)	,,
I. australiensis	±	Canis familiaris (found once) ...	,,
	0	Bettongia lusueuri (marsupial rat-kangaroo), (found once)	
I. vestitus	0	Myrmecobius fasciatus (Dasyuridae), (found once)	,,
		Diemenia superciliosa (snake), (found once)	
I. signatus	0	Phalacrocorax	Asia, N. America.
I. unicavatus	0	Phalacrocorax graculus and P. carbo. ...	Great Britain.

[1] Nymphal and larval stages are known in many of these species.

On the structure of the hypostome in ♂ Ixodes.

There are 18 species of *Ixodes* of which the males and females are known. About one of these (*pratti*, Banks) we have no information such as we require for this discussion. Of the 17 remaining species, the sexes have been found together upon the host in 13 (I (*a*) and (*b*)), and in 4 (II) only the females have been found upon the host:

I (*a*). In six the sexes have been found in copula upon the host, namely, *ricinus*, *rasus*, *ugandanus*, *schillingsi*, *pilosus* and *boliviensis*. The hypostomes in the males of the first five species are characterised by prominent basal teeth; *boliviensis* has a well-armed hypostome.

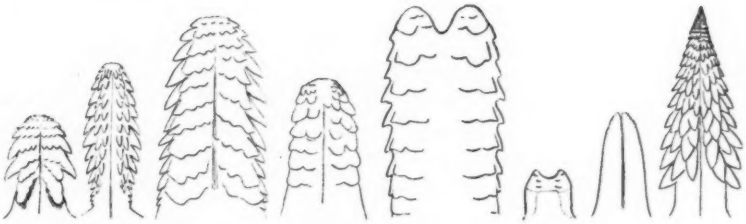
I (*b*). In seven species (*cavipalpus*, *rubicundus*, *minor*, *loricatus*, *tenuirostris*, *angustus* and *holocyclus*) both sexes have been found upon the host, but there is no specific mention of their having been found in copula. The hypostomes of the first two species possess prominent basal teeth; *minor*, *loricatus* and *tenuirostris* possess hypostomes which are well armed with pointed teeth; in *angustus* and *holocyclus* the hypostome is only moderately armed.

II. In four species (*hexagonus*, *canisuga*, *putus* and *vespertilionis* there is no record of the males having been found upon the host. In the first two the hypostome is but moderately armed, whereas in *putus* and *vespertilionis* it is almost unarmed.

The structure of the male mouth-parts, therefore, bears a direct relation to the presence or absence of the male upon the host and the occurrence of the sexes in copula upon the host. Of the six species (I (*a*)) which have been found in copula upon the host no less than five possess hypostomes with prominent basal teeth. It is probable that some of the species placed in the following division (I (*b*)) will yet be found in copula when they have been more carefully observed. In Group I (*a*) and (*b*) there are 13 species enumerated in only two of which the hypostome may be described as moderately armed. In Group II, regarding which there is no record of the males having been found upon the host, the male hypostome is but slightly armed in two and practically unarmed in two species.

Figures illustrating the hypostomes of Ixodes.

The accompanying figures show the marked sexual dimorphism which is observable in some species of *Ixodes*, and also types of male hypostomes which show considerable modifications of structure.



Figs. 19—20 represent the hypostomes of *I. ricinus* ♂ and ♀. That of the ♂ may be taken as the type of a hypostome with large basal teeth as observed also in the males of *rasus*, *ugandanus*, *schillingsi*, *rubicundus*, *cavipalpus* and *pilosus*, the basal teeth being less prominent in the last two species mentioned. In *boliviensis* ♂ the basal tooth, whilst prominent, is much less developed.

Fig. 21 represents the hypostome of *I. minor* ♂, with well-developed outer files of teeth. (The hypostome of *tenuirostris* and *loricatus* approximate to the type of structure found in the females.)

Fig. 22 represents the hypostome of *I. holocyclus* ♂. It is poorly but relatively better armed than that of *canisuga* which follows.

Fig. 23 represents the hypostome of *I. canisuga* ♂, poorly armed, and much emarginated distally. That of *hexagonus* is similar but not emarginated.

Fig. 24 represents the hypostome of *I. putus* ♂, practically unarmed, emarginated, and so reduced in length as to be useless for attaching the tick to a host. This structure alone suggests that the male of this species is probably incapable of sucking blood.

Figs. 25—26 represent the hypostomes of *I. vespertilionis* ♂ and ♀; that of the ♂ practically unarmed; that of the ♀ illustrating the dimorphism existing between the sexes.

The figures are chosen from the series illustrating *Ticks*, Part II (in press), without regard to the magnifications employed.

N.B.—It is a striking characteristic of *hexagonus*, *canisuga*, *putus* and *vespertilionis* males that the capitulum is *much smaller* compared to the size of the body than in any other male *Ixodes* that are known. The size of the capitulum apparently bears a direct relation to the dentition of the hypostome and to the habits of the male with regard to copulation and parasitism on the host.

Summary.

A consideration of the facts presented in the foregoing pages appears to warrant certain conclusions which must, however, be regarded in part as provisional and subject to revision when our knowledge of the various species of ticks has become more extended. The views here expressed may prove of practical use in the study of the Ixodidae.

The *Argasidae* represent the relatively primitive type of ticks because they are less constantly parasitic than are the Ixodidae. Their nymphs and adults are rapid feeders and chiefly infest the habitat of their hosts. In certain Argasidae (*O. moubata* and *O. savignyi*) the disadvantage of their possessing an "active" larval stage has resulted in the development of an "inactive" larva, *i.e.* the young nymph being the first to suck blood. In *O. megnini* we have a considerable adaptation brought about by the difficulty there must be in the tick entering the small aperture of the ear more than once. Owing to the Argasidae infesting the habitats of their hosts, their resistance to prolonged starvation and their rapid feeding habits, they do not need to bring forth a large progeny, because there is less loss of life in the various stages, as compared to Ixodidae, prior to their attaining maturity.

The *Ixodidae* are more highly specialized parasites than the preceding. The majority are parasitic on hosts having no fixed habitat and consequently all stages, as a rule, occur upon the host. In the genus *Ixodes* we find an adaptation of certain species according to the habits of the hosts upon which they are parasitic. In the species which usually occur upon wandering hosts both sexes are found upon the host, whereas in other species which occur on hosts possessing more or less fixed habitats the males are rarely or never found upon the host. The males of species of *Ixodes*, both sexes of which occur upon the host, are characterized, as a rule, by the possession of hypostomes with prominent teeth, the reverse being the case in forms wherein the males do not occur upon the host. Where species occur upon a wandering host it is essential, for their propagation in nature, that both sexes should be carried about upon the host.

It appears to follow that the copulation of ticks upon the host is an indication of a higher degree of specialization to a life of parasitism. When copulation does not take place on the host, we have merely the retention of a primitive character as found in Argasidae. From the

fact that many species of *Ixodes* are only known to science in their female and immature stages, it would appear probable, if my theory holds, that this is merely due to the males not having as yet been sought for in the habitats of their hosts[1].

A great many males belonging to *Haemaphysalis*, *Dermacentor*, *Rhipicephalus*, *Amblyomma* (and *Aponomma*) are known, these genera of Ixodidae being rich in species. The males of *Boophilus*, *Hyalomma* *Rhipicentor* and *Margaropus*, of which there are few species, are likewise known. In all of these genera the males are found close to the females and attached to the host. *These genera are chiefly parasitic on animals with wandering habits*, and the males of these genera do not exhibit hypostomes which are markedly dissimilar to those of the females; the hypostomes are well armed in both sexes. It is only in the genus *Ixodes* that we find well-marked sexual dimorphism in respect to the structure of the hypostome.

[1] As a further confirmation of the views expressed in this paper, I would state that we have recently (March, 1911) discovered a male of *Ixodes hexagonus* in the nest of a hedgehog. This is the first male we have captured, whereas we possess hundreds of females, nymphs and larvae found on various hosts. The rarity of the male is rendered evident by the fact that there is not a single specimen of a male in the Museums in London, Paris and Berlin.

THE PERSISTENCE OF *BACILLUS PYOCYANEUS* IN PUPAE AND IMAGINES OF *MUSCA DOMESTICA* RAISED FROM LARVAE EXPERIMENTALLY INFECTED WITH THE BACILLUS.

By A. W. BACOT, F.E.S.,

Loughton, Essex.

In the course of an inquiry into the relation between the bionomics of certain insects and epidemic disease upon which I am engaged, the following question suggested itself to me. In the event of a larva becoming bacterially contaminated by means of its food, is it possible that the resulting pupa and imago will still be infected? The only previous work bearing upon this question with which I am acquainted is that of Faichnie[1], who writes:

"On Aug. 12th, 1909, three ounces of faeces, containing *B. typhosus*, were thrown on a box of earth and covered with a wire cage and about thirty flies were let loose inside. These flies all died in a day or two, but on Aug. 26th one fly hatched; on Aug. 27th 12 flies hatched. On this same day, after the flies were hatched, the box of earth was replaced by an earthenware plate which had previously been washed in a solution of 1 in 500 perchloride of mercury: sugar and water as food in separate porcelain saucers were also introduced and the wire cover was changed for a bell-shaped mosquito net. On Aug. 26th one fly one day old was transfixed with a red-hot needle after chloroforming it, flamed, and put into a bottle of sterile salt solution. It was shaken up and 1 c.c. of this solution was put into McConkey broth, which remained unchanged in 48 hours. After this the fly was crushed with a sterile glass rod and a drop plated; *B. typhosus* was found. On Aug. 27th four flies, each one day old, were singed and examined in the same way. The control in McConkey was negative, but from the crushed flies *B. typhosus* was separated. This process was repeated on Sept. 3rd, 6th, 10th, and 13th, twice all but one of the last named gave

[1] Faichnie, N. (1909). *Bacillus typhosus* in flies. *Journ. Roy. Army Med. Corps* XIII. 580–585, 672–675.

a positive result. A second series was carried out with faeces containing *B. paratyphosus A* which also gave positive results."

Although these results are perfectly consistent with the safe passage of bacilli (ingested by the larvae) through the metamorphosis they do not establish the hypothesis, since the experimental conditions were such that the newly-emerged flies might have re-infected themselves by feeding on the contaminated material. There is no evidence in the paper that the author separated larvae which had fed on infected material and examined the pupa or imagines under conditions which would exclude the possibility of re-infection.

The experiments about to be described were directed to the solution of the problem and the results seem fairly conclusive. The species of fly used was *Musca domestica* and *B. pyocyaneus* as the infecting agent, the reasons for choosing this bacillus being that it is not highly pathogenic, and is easily recognisable.

About an inch of dry silver sand was placed in a one-pint card cream jar. A mixture of cooked food, consisting of baked milk, rice pudding, custard, boiled potato, gristle of meat, was chopped up, and, together with the contents of several agar tubes of pure cultures, of *B. pyocyaneus*, was placed on the sand, ova of *Musca domestica* being added. By the time the larvae were from half to two-thirds grown, the food was exhausted, so a small quantity of lean uncooked beef was minced, wetted with distilled water, mixed with the contents of a tube of pure *B. pyocyaneus* and allowed to remain at 29° C. for three or four hours. It was then given to the larvae. When the larvae were full-fed all remnants of food were removed and some clean sand added at the bottom of jar.

(*a*) *Experiments on Pupae*[1].

1. Five pupae were placed in a 5% solution of lysol for five minutes and then washed in sterilized distilled water. Three of these were shaken up in a tube of sterile broth for one minute, then removed to a fresh tube of broth in which they were torn up with sterilized needles. The tubes were incubated at 29° C.

The first remained sterile, the second became turbid in 11 hours, producing an apparently pure culture of *B. pyocyaneus*.

[1] Strictly speaking, the case in which the adults of the Muscidae develop is not a pupal case but a puparium. It really consists of the larval skin which is not, as usual, moulted but shrinks and becomes hardened by a secretion. The pupal skin itself is of very delicate texture. The connection between the pupa and the outer air is by way of two semicircular openings at one end of the puparium, these openings are converted into efficient filters by an elaborate series of projecting papillae and filaments that would effectively prevent the ingress or egress of fluid for some time or until some suction of air or expulsion of gas took place.

The remaining two pupae were shaken up in a tube of broth and allowed to remain in it 25 minutes, then transferred to an agar slope upon which they were broken up with sterile needles. Tubes were incubated at 29° C. The broth remained sterile, the agar showed growth in 11 hours of a pure culture of *B. pyocyaneus*. The inference is that the interior of the pupa is certainly infected.

The results of this experiment have been forwarded to Dr J. G. Ledingham, who has kindly verified them; he has also performed similar experiments with pupae supplied by me, the results of which are appended.

2. Eight pupae were placed in 3 % lysol, shaken up and allowed to remain in the solution for five minutes; they were then washed in sterilized distilled water. Two were placed in clean silver sand in a clean tube to test if sterilization had killed them. One fly subsequently emerged.

Three were placed in a tube of broth, torn up with sterilized needles and incubated at 24° C.

The broth was turbid in 13 hours and the growth again proved to be *B. pyocyaneus*[1].

The remaining pupae were placed in a tube of broth and allowed to remain at 24° C. for 13 hours. They were then removed to a fresh tube of broth in which they were broken up with sterilized needles; both tubes were incubated at 29° C. The first tube remained clear for 12 hours after the time the pupae were removed from it but showed slight turbidity in 20 hours.

The second tube became turbid in nine hours.

These results suggest either that external sterilization was not complete or that during the lengthy period of soaking in broth some passage between interior and exterior was effected. That the interior was infected seems clear from the greater rapidity of growth that followed the breaking up of the pupae.

3. Four pupae were placed in 10 % lysol for seven minutes, washed in sterilized distilled water, placed in a tube of broth and allowed to remain in it 13 hours at 24° C. They were removed to a second tube and broken up with sterilized needles. Both tubes were then incubated at 29° C. The first tube remained clear for 12 hours but showed slight signs of growth in 24 hours. The second tube showed strong growth in 12 hours.

The same remarks apply to this experiment as to Exp. 2.

4. Seven pupae were passed five or six times through a Bunsen flame on a platinum loop.

Three of these pupae were then placed in broth at 24° C. for 13 hours, after which they were transferred to a similar fresh tube and broken up with sterile needles. Both tubes were incubated at 29° C.

[1] In all cases the growth obtained appeared both culturally and microscopically to be pure *B. pyocyaneus* except that some nine slides showed slight contamination with *B. prodigiosus*.

The first tube was clear after six hours but showed signs of becoming turbid in nine hours and were distinctly cloudy in 12 hours. The second tube was markedly turbid in nine hours.

The remaining two pupae were similarly treated and gave an identical result.

These experiments seem to prove that either the pupal gut or the inner surface of the puparium is contaminated.

In view of the experiments about to be described it seems certain that the gut is infected but it may very well be that the puparium which contains the moulted portion of the larval gut is also to some extent contaminated, which would explain the slowness of growth in those controls which showed infection, since some passage of the broth might take place through the air passage after a lengthy soaking.

(*b*) *Experiments on Adult Flies.*

1. Several pupae were sterilized in 3 % lysol for two minutes and then placed in clean sand in a clean tube, the object being to prevent any possibility of the flies on emergence becoming infected by sucking at the moist infected sand of the stock-pot or the *outside* of the puparium.

Four flies taken from the above tube were placed in 5 % lysol for five minutes, then placed in a tube of broth, shaken up and allowed to remain in it for five minutes; then they were removed to a second tube and torn up with sterilized needles. Both tubes were incubated at 29° C. In 12 hours there was no result, in 22 hours the second tube was turbid; after a further period of 12 hours the first tube also showed signs of growth and finally became turbid.

2. Four flies from the same tube were placed in 5 % lysol, shaken up, and allowed to remain in the solution for seven minutes, they were then washed in sterile distilled water and transferred to a tube of broth in which they were shaken up and allowed to remain for five minutes; they were then taken out, put into a second tube, and torn up with sterilized needles. Both tubes were incubated at 29° C. In 12 hours there was no result, in 22 hours the second tube was turbid; the first remained sterile.

3. Two pupae were kept in 10 % lysol for seven minutes, then placed in clean sand in a clean tube.

One fly emerged and was placed in 5 % lysol for three minutes, removed to a tube of broth, shaken up, and allowed to remain in the solution for eight minutes, then put into a second tube and torn up with sterilized needles Both tubes were incubated at 29° C. In 22 hours the second tube produced a culture; the first remained sterile.

4. A number of pupae were taken from the general stock and placed on a sheet of paper. From one of them a fly emerged while under observation. It had no opportunity of sucking and was at once placed in 5 % lysol for four minutes, and then transferred to a tube of broth and shaken up. It sank to the bottom of the tube and was allowed to remain there for five minutes; the fly was then placed in a second tube of broth after having been torn up with sterilized needles. (*Note:*

having had no time either to expand its wings or void the fluid waste products of the pupal period, the semi-fluid contents of its body were greater in volume and differently coloured from those of the other flies experimented on. It was no doubt owing to the fact that its body was still wet and that it had not undergone the normal purge, that it sank while in the control tube.)

The first tube remained sterile, the second showed a *strong growth of B. pyocyaneus in* 12 *hours.*

The slowness of growth in those of the controls which were infected compared with the *rapid* development from crushed pupae points to some slight passage between the interior of the puparium and the surrounding fluid when the insects were allowed to remain in broth for such lengthy periods as 13 hours. The view I have expressed as to the cause of slow and late cultural development being due to different sources of infection is supported by the later experiments on flies and there seems to be no doubt but that the *pupal gut is heavily infected.* With the adult flies the suggestion is that they are infected internally on emergence. The rapidity of the growth from the freshly emerged specimen, which had had no opportunity of voiding the contents of the gut, compared with those that had this opportunity, points clearly to the infection of the gut and affords additional support to the correctness of this conclusion in regard to the pupa.

The very rapid development of the growth in the control experiments with "fired" pupae may have been due to the heat producing an outward current of air or vapour through the air passages, so that when the insect was plunged into cool broth immediately after "firing" an inward suction of the media occurred owing to the partial vacuum set up. It is also possible that the pupae used in Exp. 4 were actually ruptured by the heating process, since three others treated at the same time flew off the platinum loop during the firing process, a fact which suggests that a considerable vapour pressure was developed within the pupa case[1].

Quite apart from the suggestiveness of this result and its bearing upon my special line of research, the fact that organisms ingested by the larva with its food may persist throughout the pupal stage and still be present in the adult insect is of importance. It is of significance in connection with typhoid and infantile diarrhoea, as the possibility of the larvae being reared in infected excreta may render the danger acute.

[1] Later experiments show that if the ends of the puparia are varnished, so as to block the stigmata, that after being allowed to soak in 10 % solutions of lysol or formalin for periods up to 39 hours and then in control tubes of sterile broth for further periods up to 14 hours, the pupae will still give a positive result when cracked or pierced in the culture tube. The control tube remains sterile.

Moreover if it is true, as Sharpe supposes, that *Musca domestica* hibernates as a pupa, the bacteria must find an asylum in the pupal fly during those periods when these diseases in man are in abeyance.

I may add that I am engaged in similar experiments with fleas, the results of which I hope to publish in due course.

Conclusions.

1. Pupae and imagines of *Musca domestica* bred from larvae infected with *P. pyocyaneus* under conditions which exclude the chance of re-infection in the pupal or imaginal period undoubtedly remain infected with the bacillus.

2. In the imago the infection is maximal at emergence and then diminishes suddenly.

3. The possibility of a dangerously pathogenic micro-organism being taken up by the larva and subsequently distributed by the fly is one which deserves serious consideration.

ADDENDUM.

By J. C. G. Ledingham.

(*Lister Institute of Preventive Medicine, London.*)

As Mr Bacot points out, the great difficulty in these experiments is the efficient disinfection of the pupal exterior before testing the bacterial content of the interior. There is a strong probability that some of the growth-effects immediately after disinfection are due not to organisms still present on the outside of the pupae but to organisms from the interior which have gained access to the broth during the shaking-up process following disinfection.

Of those experiments in which disinfection of the pupae was carried out by means of 5 % lysol acting for ten minutes, two were unsatisfactory as the broth tubes in which the disinfected pupae had been washed showed growth. From the luxuriant cultures obtained on the agar slopes inoculated with the mashed-up pupae, however, there could be no doubt that the interior of the pupae was always heavily infected.

In the last experiment performed with pupae sent me by Mr Bacot another method of disinfection was employed. After washing the pupae in successive tubes of broth and saline solution, they were transferred to a Petri dish containing a small quantity of absolute alcohol, where they remained for three to four minutes. The alcohol was then ignited and allowed to burn out almost completely. Some of the pupae, as the result of the process, seemed to be slightly desiccated

externally. They were then placed in 10% formalin for four to five minutes. Thereafter they were removed one by one to a tube of sterile broth and shaken up. From this tube they were transferred to a second broth tube and shaken. Finally from this second tube each pupa was removed to an agar slope and mashed up with a strong platinum loop. The two broth tubes and the series of agar slopes were then incubated at 37° C. Next day both the broth tubes were sterile but *all the agar slopes showed abundant growth in which B. pyocyaneus was present.* After 48 hours the broth tubes were still sterile but on the third day one broth tube (the second) showed growth. This slight discrepancy, however, in no way invalidates the general conclusion arrived at by Mr Bacot that the pupal interior may become heavily infected by organisms taken up during the larval stage. By further improvements in technique it may be possible to eliminate entirely any growth-effects before the final mashing-up process[1].

EDITORIAL NOTE.

Dr Graham-Smith, who has been carrying out investigations for the Local Government Board, has obtained some very interesting results.

Blow-fly (*Calliphora erythrocephala*) maggots were fed on meat infected with the spores of *B. anthracis.* When full fed the maggots were transferred to clean cages in which they pupated. The flies emerged about three weeks after the larvae had fed on the infected meat. Cultures were made from the organs of 51 of those flies, in some cases shortly after emerging, and in some cases when the flies were a few days old. *B. anthracis* was obtained from 26 flies. *B. anthracis* was also obtained from agar plates on which the flies had walked, from faeces deposited by them, and from syrup on which they were allowed to feed. Several of the cultures obtained were proved to be virulent.

These investigations will shortly be published in *Reports to the Local Government Board on Public Health and Medical Subjects.*

G. H. F. N.

[1] Since this was written I have devised a simple but efficient method of examining the bacterial content of the pupal interior in *M. domestica.* The pupa is held lightly between the left thumb and forefinger so that its blunt extremity is free. With a small searing-iron this extremity is seared and at the same time flattened. It is then pierced by a fine capillary pipette controlled by a rubber teat. The pupal contents are stirred up by the extremity of the pipette and finally drawn up into the tube whence they are immediately squirted on to plates. The whole process takes less than a minute to perform.

Recently I have been successful in isolating *B. typhosus* from pupae the larvae of which have fed on this organism. The experiments will be published shortly.

THE EMBRYOS OF *FILARIA LOA*.

By OTTO V. HUFFMAN, M.D.

(*From the Laboratory of the Cincinnati Hospital, Cincinnati, Ohio, U.S.A.*)

(With Plate IV.)

THE embryo of *Filaria loa* has never been fully studied or described and for this reason the hypothesis put forward by Manson, that the *Filaria diurna* is the larva of *Filaria loa*, remains unproven. When Manson suggested this connection he had at hand some sketches of embryos which Leuckart had made and sent to him. As these sketches were not of sufficient aid to establish the identity of the embryos with the larvae found by him in the blood, and as no metamorphosis takes place in the larvae while circulating in the blood, it is reasonable to suppose that they must have been rather crude sketches. However, the hypothesis came to be accepted because it was well supported by the fact that the two worms have the same geographical distribution and also by the occasional discovery of both in the same host. At the same time that this hypothesis was advanced, Manson changed the name from *Filaria sanguinis hominis major* to *Filaria sanguinis hominis diurna* and said that the species was practically indistinguishable on morphological grounds from the well-known *Filaria sanguinis hominis* of Lewis (*Filaria nocturna*, Manson) *i.e. Filaria bancrofti*. This fact made it all the more necessary to observe embryos taken from an adult female *Filaria loa* before venturing to state the origin of the larvae found in the blood.

Only one author has stated that *Filaria loa* is the adult form of the *Filaria diurna* of Manson and he had nothing new in the way of evidence

to justify him in making such a statement. This was Brumpt who had observed in the blood of a patient a microfilaria which he had described as a new species, *Filaria bourgii*. Upon the death of this patient he found in the heart a portion of a female worm without head or tail, but presenting cuticular bosses like those of *Filaria loa*. After returning to Paris he compared his specimens of microfilariae with those described by Manson as *Filaria diurna* and he decided that they were identical, and the mere association of these microfilariae with an adult *Filaria loa* in the same host led him to make the above statement.

In 1906 Livon and Pénaud observed some embryos of *Filaria loa* which had been artificially removed from an adult female, but their description is quite brief and unaccompanied by any figures.

Recently Burrows has published an article on the relation of *F. diurna* to *F. loa*, but both of the adult *F. loa* removed from his patient were mutilated and therefore the opportunity of obtaining embryos from the female was lost.

I have been more fortunate than my predecessors in being able to observe and study a live female *F. loa*, fully matured, with uteri filled with ripe embryos. These embryos have the same dimensions as those recorded for *F. diurna* and are identical with them in other respects.

Since Huffman and Wherry have already described the anatomy of the adult *F. loa* (see this Journal p. 7), I take this opportunity of adding a few notes in regard to the living adult from which the embryos were obtained.

Dr Emil Blunden brought to the laboratory a live female *Filaria loa* 62·5 mm. in length which he had just removed from the eye of Mrs B. This is the eighth specimen removed from Mrs B. The worm had been placed in warm tap water, and there was no doubt about its being alive because it continually performed all manner of movements. The worm was very white, the cephalic extremity and the tail, into which the genital organs did not enter, were translucent. The intestine, being black, was quite visible throughout its course.

The worm was placed on a large glass slide so as to be observed under the microscope. The cephalic papillae were easily discerned but did not project from the cuticle as much as their granular contents projected from the musculature. The oesophagus was long and narrow and not bordered by such big cylindrical cells as in preserved specimens; moreover it was clear and appeared to have narrow culs-de-sac opening into it from either side. The viscera occupied less than two-thirds of the diameter of the body and the musculature was clear and translucent.

The distance from the tip of the head to the most anterior loop of the oviducts was about twice as great as in preserved specimens. As the worm became partially dry the cuticular bosses on top became visible as warty plaques like those represented in Blanchard's well-known drawing. This circumstance accounted for his description which we were previously unable to reconcile with the neat transparent bosses observed by us in preserved specimens. The tail was carried well curved with a slight spiral twist.

In order to keep the worm active, the warm water in which it was kept was frequently changed. After examining the structure of the parasite we decided to try some experiments to see if we might cause the birth of some embryos. With the posterior two-thirds of the worm in warm water and the anterior third projecting on to the dry portion of the slide, we covered the anterior extremity, as far back as the vulva, with a large drop of fresh human blood. The worm immediately gave evidence of distaste for the blood by withdrawing its head and moving away. An attempt to produce abortion by pressure and by irritation was then made, but without avail. Then the worm was placed over night in a small phial of warm water in an incubator at 33° C. The next morning the worm was quite inactive, and upon examining the water no ova or embryos were found. Many bacteria were present as the water had not been sterilized nor had the worm been handled in an aseptic manner on the previous day. I decided to section the body near the vulva to obtain embryos before death supervened. One could see the uteri which were intertwined with the loops of the oviducts, they were distended with fully developed uncoiled embryos packed in bundles end to end.

As the body was divided transversely with a sharp scalpel, myriads of coiled embryos in their sheaths and ova escaped into the drop of water on the slide. The embryos were alive, as shown by their swinging their anterior one half from side to side or by lifting the anterior half upwards. They were not locomotive. No spines or lips were seen. No forward and backward motion within the sheath occurred, except as it might be caused by outside pressure. I observed coiled embryos start the process of uncoiling (see Plate IV, Fig. 1). This seems important as Manson has held that the sheath of the coiled embryo is the same as the larval sheath observed in the blood while Ludwig and Loos believe that this sheath is discarded before birth. Livon and Pénaud observed the disappearance of the egg envelope and the birth of the

embryo with its sheath. We observed some of these coiled embryos uncoil and stretch the sheath which contained them.

Of these thousands of embryos in water on the glass slide we made a number of fixed preparations to be variously stained. Others were placed in wet human blood preparations sealed with vaseline.

Some were injected into the subcutaneous tissues of a guinea-pig. The water containing some of them was given to another guinea-pig to drink. The guinea-pigs died recently but no cause of death could be discovered.

I did not succeed in getting the embryos to butt their way out of their sheaths after heating a blood preparation which had been chilled on ice as described by Manson. Nor was I successful in reviving embryos which had been dried as suggested by the same author. In heating a wet blood preparation slight currents may be set up which cause the appearance of slight motion on the part of the embryo and give the appearance of butting.

Measurements :—

Coiled embryos 0·051 × 0·036 mm.
Embryos 0·006 × 0·3 mm.
Average projection of sheath anteriorly 0·009 mm.
,, ,, ,, ,, posteriorly 0·009 mm.
Anterior V spot 0·09 mm. from head end.
Posterior ,, 0·06 mm. from tail end.

Livon and Pénaud give the length as 0·3 to 0·35 mm. and the width as 0·005 to 0·007 mm. The maximum diameter of the embryos, 0·006 mm., is maintained with very slight diminution until a point 0·24 mm. from the head end is reached where the body commences to taper to the posterior extremity. The posterior extremity is rounded. It is not sharply pointed and we cannot conceive such a tail being folded upon itself within the sheath as described in the table given by Neveu-Lemaire. At the anterior extremity, which is broadly rounded, there are two highly refractile black specks. 0·09 mm. from the head end is the ovoid mass which presents the anterior **V** spot. This ovoid mass causes a slight bulging of the body on three sides but not on the fourth as it does not extend deeper than the plane of the central longitudinal axis of the body. Within this clear highly refractile mass there are two black granular specks separated sufficiently to give rise to the **V** (spot) which is an optical effect. The point of the **V** is directed to the periphery. 0·06 mm. from the tail end is a similar but smaller ovoid

body and V spot. When the ovoid masses are seen from a direction midway between that which shows their broadest dimensions and that which shows their least dimensions, a conical papilla is observed which has its apex directed laterally (Plate IV, Fig. 3). All of the embryos have these V spots and they are not properly situated in relation to the length of the embryo to represent the vulvar and anal openings as suggested by Manson and by Ashburn and Craig.

In the stained specimens the ovoid masses at the V spots did not cause a bulging as in the live specimens, but at their site there was a slight narrowing of the body. Throughout the body in most specimens we could determine the stained intestine. Behind the posterior V spot the intestine was not stained perfectly but in sections gave rise to the appearance of rod-like bodies placed end to end as described by Ashburn and Craig. These latter authors permitted an ellipsis in their article where they state "At about the middle of the posterior of the worm are situated the posterior V spot and the papilla." They attempted to describe a new species of Filaria but did not point out any determining morphological characteristics. The microfilaria which they described as a new species, *F. philippinensis*, had been previously diagnosed in their case as *F. diurna*.

The interior of the live embryos, with the exception of the refractile specks at the head and the two ovoid masses, is entirely homogeneous. In the specimens which had been stained after death, the interior was made up of irregular shaped masses which stained deeply (Plate IV, Fig. 4); the ovoid masses were not so large. The posterior ovoid mass becomes stained before the anterior mass which is more resistant to staining solutions. Embryos stained by a very dilute solution of basic fuchsin, while alive, take up the stain at the periphery of the ovoid masses and in the specks near the head end (Fig. 2). They also, if stained by a stronger solution, take up the stain in the rudimentary intestine (Fig. 4). The unstained live embryos are white and translucent. Those which were placed in human blood (wet preparations) appeared of the same colour as the blood plasma. None of the embryos either in water or blood lived more than an hour after separation from their mother. As the measurements of *Filaria diurna* do not exceed those of the embryo of *Filaria loa*, as measured by Livon and Pénaud and by myself, it is fair to assume that no great changes take place while in the blood and that a study of viviparous embryos should give results not unlike those of a study of the filaria found in the blood.

Foran, who found *Filaria diurna* in the blood of 12·5% of 826 natives of South Nigeria, noted an outflowing of substance at the anterior V spot. This may have been due to weakness at this point for Ashburn and Craig noted the same in some of their specimens. In my specimens there is a distinct bulging of the ovoid body at the anterior V spot, yet it cannot be represented to be so large as in the sketch of Foran.

Livon and Pénaud noted that some had blunt tails. Many years ago O'Nielly and O'Neil observed microfilariae which had two specks at the head, alimentary canals, "bluntly pointed" tails, and which died shortly after being placed upon the glass slides.

Some of the embryos which I had left sealed in water were examined two weeks later. Degeneration had set in; besides the two V spots, which did not show up so clearly, there were other spots which caused some confusion as they were not so distinct, nor were they in focus and highly refractile at the same time as the known V spots. The lines and anatomy were not so clearly defined as in the fresh living embryos and it is easy to see why the filariae found in the blood have been variously described. When all of the various preparations were examined one month later it was found that the sheaths of the specimens stained by fuchsin were no longer discernible; that the embryos in the wet preparations presented irregular masses in the anterior portion of the body as well as several vacuoles which might be taken to be V spots; that the ovoid masses were no longer easily distinguishable; and that the tails had lost their "plumpness," being flabby and drawn out posteriorly, and in some preparations which had been disturbed they were folded upon themselves. The sheaths of some of the embryos in the wet preparations were collapsed and extended as a linear projection fore and aft. Hence it may be seen that it is impossible to determine any definite morphological characteristics except in fresh embryos, which present a definite form, granular specks at the head, alimentary canal, and two ovoid masses definitely situated and representing without doubt the beginning of undifferentiated organs.

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DESCRIPTION OF PLATE IV.

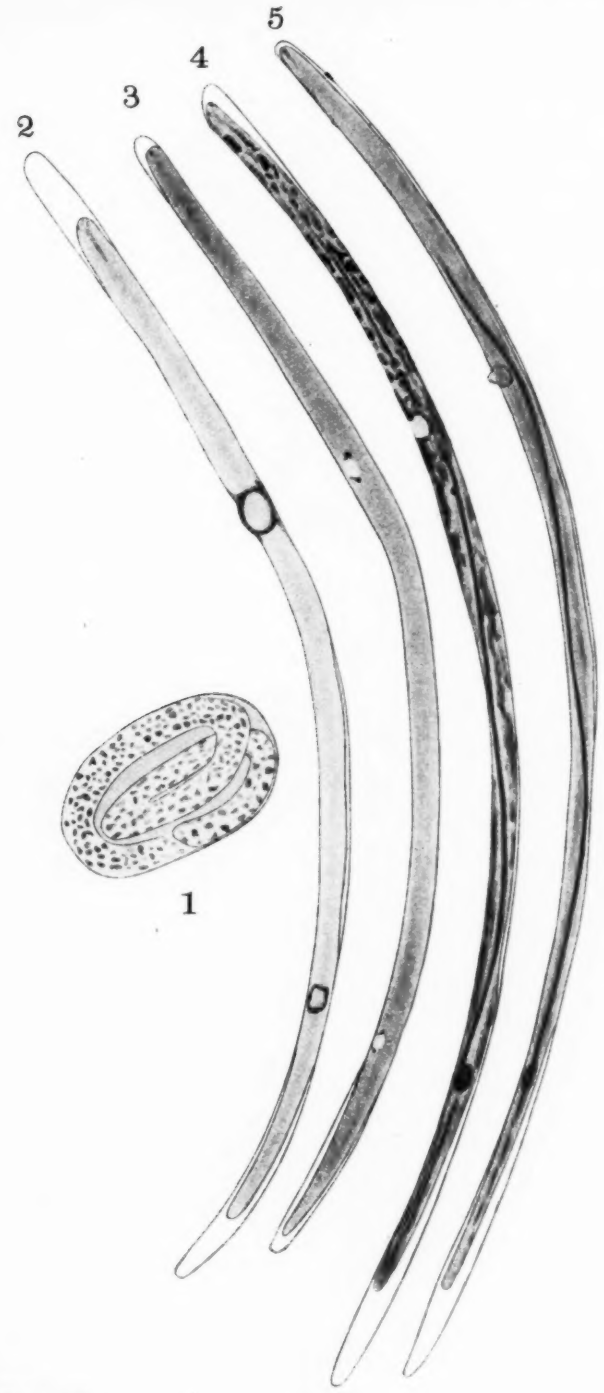
Fig. 1. A coiled embryo observed in the process of uncoiling and stretching the sheath. The irregular masses throughout the body of the embryo and deeply stained by fuchsin were not observed while the embryo was alive.

Fig. 2. A mature embryo stained with a very dilute solution of fuchsin while alive. Some little granular masses at the head end, the periphery of the ovoid masses at the anterior and posterior V spots are the only portions that have been stained.

Fig. 3. A mature embryo as seen in a wet blood preparation while alive. Two black specks at the cephalic extremity. A highly refractile conical papilla and ovoid mass with two dark specks separated sufficiently to cause the appearance of a V situated 0·09 mm. from the head end and another but smaller ovoid mass, papilla and V situated 0·06 mm. from the tail end.

Fig. 4. A mature embryo stained with a stronger solution of fuchsin. Irregular masses throughout the body, due to post-mortem changes, have been stained. The anterior ovoid mass has not taken up the stain whereas the posterior ovoid mass has. The intestine is seen throughout part of its course.

Fig. 5. An embryo which did not exhibit the irregular masses shown in Fig. 4. The rudimentary intestine is deeply stained.



O. V. Huffman, del.